REMARKS

A check for the requisite fees for a five-month extension of time and for filing a Request for Continued Examination (RCE) accompanies this response. Any fees that may be due in connection with filing this paper or with this application during its entire pendency may be charged to Deposit Account No. 06-1050. If a Petition for extension of time is required, this paper is to be considered such Petition, and any fee charged to Deposit Account No. 06-1050.

Claims 50-52 and 73-115 and 117-128 are pending in this application. Claims 114 and 115 are amended for clarity, and claim 116, which is duplicative, is cancelled without prejudice or disclaimer. Basis for the amendments can be found in the claims in the original parent, as well as in the instant application and intervening parent application. No new matter is added.

An unexecuted Declaration of Stephen Fabijanski (herein DECLARATION 5) pursuant to 37 C.F.R. §1.132 accompanies this response. An unexecuted Declaration of Gyula Hadlaczky (herein DECLARATION 6) pursuant to 37 C.F.R. §1.132 accompanies this response. The executed Declarations of Stephen Fabijanski and Gyula Hadlaczky will be provided under separate cover upon receipt. As noted in the Declaration of Fabijanski, Dr. Fabijanski is the Director of Research and Development of Agrisoma, which was a spin-out company from Chromos Molecular Systems, to commercialize the plant satellite artificial chromosomes. Agrisoma is a licensee of the application at issue. Also, as noted in the Declaration of Hadlaczky, Dr. Hadlaczky is an inventor of the application, one of the founders of Chromos Molecular Systems, and an employee of the BRC, a joint owner of the instant application.

All Responses and Declarations of record, including the Response and Declaration 1 of Fabijinski filed July 16, 2003; the Response and Declaration 2 of Fabijanski, filed April 22, 2004; the Response and Declaration 3 of Fabijanski filed January 16, 2005; and the Response and Declaration 4 of Fabijanski filed November 09, 2005, responsive to previous Office Actions are incorporated by reference in their entirety herein.

Preliminary Remarks

Applicant acknowledges the Examiner's withdrawal of the rejection of claims under 35 U.S.C. §112, second paragraph based on the term "satellite artificial chromosome."

Further, Applicant brings to the Examiner's attention copending application Serial No. 10/287,313, which contains claims directed to methods of producing a transgenic plant.

As noted in the response mailed February 15, 2007 to the non-final Office Action in copending application Serial No. 10/287,313, Applicant requests deferral of any issues regarding obviousness-type double patenting until there is allowable subject matter. Also, Applicant reminds the Examiner that if a rejection for obviousness-type double patenting is set forth in the next Office Action, the Examiner is precluded from making the Action Final.

Before directly addressing the outstanding rejections, it is noted that the instant claims represent a pioneering invention based upon the discovery of and exploitation of a fundamental process common to all chromosomes. As described in the application, it was discovered that chromosomes undergo amplification processes that, when exploited as described in the instant application, can be employed to produce what applicant has called satellite artificial chromosomes (SATACs, which are types of ACEs). The application generically identifies the intrinsic large-scale amplification that occurs. The application teaches how to exploit this to generate satellite artificial chromosomes in **any cell** by introducing nucleic acid into cells, which nucleic acid either randomly integrates into the pericentric heterochromatin or is targeted to such region, growing the cells and looking for the amplified structures, which constitute precursors to the SATACs or SATACs, and then further manipulating cells or SATACs and/or isolating them. The application specifically teaches (*i.e.*, page 9) that the process can be applied to any species, including plants and animals, to produce SATACs. The chromosomal processes that gives rise to these amplified structures is shared among chromosomes of all species. The specification exemplifies the process with rodent cells and mammalian cells, and teaches that the process occurs in any eukaryotic cell, including human cells. Further, as shown in the application and in Declarations of record in copending and issued family members, in this application and attached hereto, this process occurs in plant cells as well as mammalian cells. As described, for example, in the attached DECLARATION 5 and 6 the amplification event(s) and production of SATACs occurs in diverse species. The DECLARATIONS 5 and 6 describes their production in diverse species, namely in human cells and in plant cells, and hence is, as taught in the application, reproducible and generic.

Further, the application also teaches how to use any SATAC produced from any cell to introduce into a plant cell to generate a transgenic plant as claimed. The specification teaches how to do this. Also, Declarations of record in this case, using methods as taught in the application, exemplify various techniques to introduce a SATAC, e.g., animal or plant

SATACs, into cells (see Declaration 1 and Declaration 3) and also the generation of a transgenic plant therefrom (see e.g., Declaration 3).

Furthermore, the pioneering nature of these satellite artificial chromosomes has been acknowledged and recognized by the US Patent Office, which granted three patents to family members of this application. These include patents with claims to SATACs per se, human SATACs, cells that contain SATACs per se, methods of producing a product in a cell containing a SATAC without reference to species of satellite artificial chromosome or cell. Issued claims cover SATACs from any species and include claims to cells that contain SATACs. Neither SATACs nor cells are limited to particular species. Issues of scope were fully vetted during prosecution of the issued patents.

In addition, as noted in the DECLARATION 6, one of the inventors named in the application, Dr. Gyula Hadlaczky, has been awarded the prestigious Széchenyi Award in 2000 in his native country, Hungary, for the work that is the subject of the above-captioned application. This evidences the pioneering nature of the claimed subject matter.

It respectfully is submitted that the Office has provided no sound basis upon which to question the universality of this discovery nor to question its own prior determination of patentability of these artificial chromosomes. By rejecting the claims in the instant application, the Office is in effect raising issues (unwarranted) regarding issued claims (see, *e.g.* U.S. Patent Nos. 6,077,697 and 6,025,155) in parent patents, which claims are entitled to a presumption of validity.

Furthermore, based upon the technology as described in the initial application, filed in April 10, 1996, Chromos Molecular, Inc. (hereinafter Chromos), a now publicly traded company, was founded. Strikingly, the methods for preparing SATACs and the resulting SATACs, as described in this application and the priority applications, remain a cornerstone of the Company's business. Chromos, in fact, has spun-off a subsidiary, Agrisoma Biosciences Inc, that is exploiting the plant satellite artificial chromosomes, which have been prepared by the methods described in the instant application. Chromos has licensed the SATACs and uses thereof to numerous entities, including large pharmaceutical companies, such as Pfizer, for gene therapy and for production of transgenic animals, for production of gene products and other applications. Numerous therapeutic genes and products have been expressed by SATACs produced in accord with the methods in the instant application and priority application in a variety of cell types, including human, bovine, avian, plant and others.

There is no evidence of record, nor could such evidence be obtained, that the inventors did not possess this technology in April 1996 (the application includes working examples), nor is there evidence that this application, which details, step-by-step methods for making satellite artificial chromosomes, does not enable preparation and use of these artificial chromosomes *in any species*. The undersigned urges that, not only are the rejections unwarranted, but maintenance of such rejections in this application and in general is damaging to the biotechnology industry and to the integrity of the patent system. The application clearly details preparation of SATACs and their use, establishes that the methods and products are universal in applicability and constitute the foundation of a company, now publicly traded in Canada. The Declarations of record establish and demonstrate that by following the teachings in the application SATACs can be generated in plants and animals and that such SATACs can be introduced into cells to produce transgenic plants and animals. The USPTO has issued claims of a scope commensurate with such disclosure, and the company and third parties have reviewed and relied upon the validity of such patents. Maintaining unwarranted rejections of subject matter previously issued by the Patent Office undermines the integrity of the patent system. Accordingly, allowance of the presently pending claims, which are within the scope of issued claims, respectfully is requested.

I. REJECTION OF CLAIMS 115-128 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 115-128 are rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement as the claims are alleged to contain new matter. Specifically, the Examiner alleges that there is no support in the specification for the term "plant satellite artificial chromosome." While claim 115 is amended to obviate this rejection, this rejection is respectfully traversed.

The specification describes in detail satellite artificial chromosomes (SATACs), and methods of making such SATACs that is generic and applicable to diverse species, including plants (see e.g., at page 8, lines 27-29; page 9, lines 14-18; page 12, lines 5-9; page 16, line 22 to page 17, line 4), and lists plants as among the species in which SATACs can be prepared. For example, page 9, lines 14-18 of the specification recites:

> Thus, methods for producing MACs of both types (i.e. SATACs and minichromosomes) are provided. These methods are applicable to the production of artificial chromosomes containing centromeres derived from any higher eukaryotic cells, including mammals, birds, fowl, fish, insect and plants.

In addition, originally filed claims recite plant satellite artificial chromosomes and methods of making satellite artificial chromosomes. Original claim 67 recites the SATAC introduced into a plant cell to generate a transgenic plant is a plant artificial chromosome. Accordingly, it is respectfully submitted that the claims do not contain new matter. To advance prosecution, however, claim 115 is amended to recite that that the SATAC is one containing a plant centromere, which, as defined in this application and the original application, is a plant SATAC.

II. The Rejection of Claims 50-52 and 73-128 Under 35 U.S.C. §112, First Paragraph

Claims 50-52 and 73-128 are rejected under 35 U.S.C. §112, first paragraph for alleged lack of written description, because it is alleged that the specification does not describe the subject matter in such a way as to convey to one skilled in the relevant art that the inventor(s) had possession of the claimed subject matter at the time the application was filed. Specifically, the Examiner urges that the specification does not adequately describe the starting material of any SATAC required to practice the claimed method. The Examiner alleges that the specification only provides guidance for use of a satellite artificial chromosome from a single mammalian species, specifically a mouse SATAC, and does not provide guidance regarding the characterization or isolation from any other animal species, such as reptiles, birds, insects, worms, or mollusks; or any of a multitude of plant species. The Examiner further urges that Applicant has not demonstrated any structural features which are conserved throughout the genus of any SATAC (animal or plant) and which are correlated with function. Notwithstanding the evidence provided in connection with the prosecution of the instant application, the prosecution of the prior issued patents, demonstrated production of satellite artificial chromosomes in other species, including humans. In the current Office Action, the Examiner also raises additional points in response to Applicant's Office Action mailed November 09, 2005. These points are set forth below and are further rebutted in detail below.

1) The Examiner states that Applicant's contention that plant SATACs do not have any particular structural requirements is contradicted by Applicant's specification, which states that "plant artificial chromosomes... refer to chromosomes that include...plant centromeres." The Examiner cites Jiang et al. (Trends in Plant Science, 8:570-575 (2003)) to show that there is no conservation of centromeric sequences among eukaryotes.

2) Responsive to Applicant's arguments regarding the presumed validity of patented claims in the parent patents, issued as 6,025,155 and 6,077,697, the Examiner states that because the Written Description Guidelines were not published in the Federal Gazette until January 2001, the instant Office Action contains a written description rejection not applied in the allowed parent applications.

3) The Examiner urges that the case for a plant satellite artificial chromosome is not persuasive because Declaration I lacks any teaching of a plant SATAC or any identifiable components of a plant SATAC. The Examiner states that the Declaration I provides no location of the satellite artificial chromosome, the size of any remaining nucleic acid sequence is unspecified, and no information is provided regarding the possible function of integration sites of such nucleic acids.

4) The Examiner urges that the relationship of the specifics of the specification figures 2 and 3 with those of the Fabijanski figures 1, 2, and 3 [in Declaration 4] is not apparent. Figures 2 and 3 show schematics of a complex molecular pathway starting with chromosome #7 being transfected with foreign DNA, which DNA is described as λ DNA, and other macromolecular complexes comprising, for example, heterochromatin and euchromatin. The Examiner states that the Figures in Declaration 4 cannot be evaluated until a detailed correlation, which includes the indicated identified area of the various panels of the figures, is provided.

5) The Examiner states that although the Declaration No. 4 appears to demonstrate the isolation of plant SATACs comprising centromeres from a single plant species, namely tobacco, the Written Description Guidelines mandate that the specification provide adequate written description as of its filing date.

6) The Examiner states that the Declaration 4 has not provided any sequence information regarding which tobacco sequences were conserved in the SATAC produced from the tobacco genomic sequences. The Examiner urges that the Applicant has not provided any structural features of a second species of the claimed genus, which would be required to demonstrate the conservation of sequence between the two species, namely mouse SATAC and tobacco SATAC.

These comments are rebutted below. The rejection of claims under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement, respectfully is traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject matter at the time of filing of the application In re Wertheim, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

35 U.S.C. §112 requires a written description of the invention. This requirement is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." Vas-Cath, Inc. v. Mahurkar, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] Vas-Cath, Inc. v. Mahurkar, at 1115, quoting In re Ruschig, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.,* whatever is now claimed. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." Ralston Purina Co. v. Far-Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting In re Kaslow, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." In re Gosteli, 872 F.2d 1008, 1012, 10 USPQ.2d 1614, 1618 (Fed. Cir. 1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. In re Wertheim, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also* Ex parte Sorenson, 3 USPQ.2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a parent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. In re Reynolds, 443, F.2d 384, 170 USPQ 94 (CCPA 1971); and In re Smythe, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Furthermore, the subject matter of the claims need not be described literally (i.e. using the same terms or inhaec verba) in order for the disclosure to satisfy the description requirement. If a claim is amended to include subject matter, limitations, or terminology not present in the application as filed, involving a departure from, addition to, or deletion from the disclosure of the application as filed, the examiner should conclude that the claimed subject matter is not described in that application. This conclusion will result in the rejection of the claims affected under 35 U.S.C. 112, first paragraph – description requirement, or denial of the benefit of the filing date of a previously filed application, as appropriate.

The claims

Independent claim 92 recites:

> A method for producing a transgenic plant, comprising introducing a satellite artificial chromosome (SATAC) into a plant cell; and
> growing the plant cell under conditions to produce a transgenic plant.

The dependent claims recite particulars of the SATACs or methods.

Analysis

The Office Action alleges that there is a lack of written description of the starting material of genus of SATACs for use in the claimed methods of using any satellite artificial chromosome or any plant artificial chromosome. It is alleged (without providing any support therefor) that plant and animal chromosomes function differently and that it is unclear whether plant and animal chromosomes and centromeres have appreciable sequence conservation of any conserved domains. Applicant respectfully disagrees.

As discussed below, and highlighted in the attached DECLARATION of Hadlaczky, the process by which SATACs are generated derives from an amplification process common

to all eukaryotic chromosomes. While the Examiner may state that plant and animal chromosomes "function differently," with respect to the amplification events that can be exploited to produce SATACs, the processes are universal. Hence, the unsupported statements by the Examiner are not relevant, since they do not apply to the claims at issue. The universality of the processes besides being described in the application, are demonstrated in the DECLARATIONs of record and those attached hereto. As described in the application, introduction of a DNA fragment into the pericentric region of the chromosome initiates amplification events that produce a dicentric chromosome and ultimately the formerly dicentric chromosome from which SATACs can be generated. Introduction of a DNA fragment into the pericentric heterochromatic region of a chromosome can occur by chance, or, can be targeted, such as in the DECLARATIONs of record (the 1996 sequence was not used for targeting, but encodes an APS sequence that is not relevant to the issues under consideration). The Declarations of record and those provided herewith show that the same events occur in plants and animals when giving rise to a SATAC. Hence, as described in the application, the process is universal.

The Application is Replete with Detailed Description, Working Examples and Figures to Evidence Possession of Satellite Artificial Chromosomes Generically

As provided for by applicable law and restated in the "Guidelines for Examination of Patent Applications Under 35 U.S.C. §112 ¶ 1, 'Written Description' Requirement (66 FR 4, 1099-1111 (January 5, 2001) (hereinafter the Guidelines for Examination under 35 U.S.C. §112, first paragraph), possession may be shown in many ways, including, for example, by (1) describing an actual reduction to practice of the claimed invention, (2) a clear depiction of the invention in detailed drawings or in structural chemical formulas or (3) any description of distinguishing identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed subject matter. It is respectfully submitted that the possession of satellite artificial chromosomes, and plant satellite artificial chromosomes in particular, is amply demonstrated in all three of these exemplary ways by the instant application.

The application describes a universal process for the generation of SATACs. As described in the application, introduction of nucleic acid into a cell, which gets incorporated into a chromosome initiates amplification events, leading to the generation of a *de novo* centromere, resulting in intermediate chromosome structures such as a dicentric chromosome and sausage chromosome, and ultimately a SATAC. Introduction of the DNA fragment

under conditions in which the amplification event will be observed, such as growth under selective conditions, is all that is required to initiate the process. Introduced nucleic acid will, by chance incorporate into the heterochromatic pericentric region of a chromosome; or the probability can be increased by targeting it, such as by inclusion of rDNA in the fragment.

The application provides working examples of the generation of SATAC using mouse cells, but describes that the phenomenon as generalizable and applicable to other eukaryotic cells (see e.g., at page 30, lines 9-12; and at page 36, lines 3-4). The Declaration 6 of Hadlaczky further evidences the universality of the process of generating SATACs. As described in the DECLARATION 6, the processes underlying the generation of SATACs is fundamental to all eukartyotic apecies, and is as described in the application. Hence, as set forth in detail below and in the DECLARATION 5 of Fabijanski provided herewith, **no knowledge of the plant centromere sequence is required to produce a SATAC or to use a SATAC in the methods as claimed**. As taught in the above-captioned application introduction of a DNA fragment into the heterochromatic pericentric region of a chromosome leads to an amplification event, resulting in *de novo* generation of a centromere. This is described in the application and also exemplified in DECLARATION 5. SATACs were generated following amplification and the generation of a *de novo* centromere of the chromosome in which hit is introducerd.. No knowledge of a plant centromeric sequence nor any sequence was required. For convenience, the fragment introduced included rDNA to target it to pericentric heterochromatin. As described in detail below, the resulting SATACs, including SATACs produced from animal and plant chromosomes, are produced in the same manner and undergo the same amplification events through the same types of intermediates.

As discussed more below and previous, the application provides a detailed description of preparation of SATACs, including drawings and schematics, detailed description and working examples. Furthermore, SATACs are generically claimed in the originally filed application and are generically claimed in issued patents, evidencing the Office's determination that applicant had possession of a broader genus. Hence, it respectfully is submitted that the specification provides sufficient identifying characteristics of satellite artificial chromosomes, including plant artificial chromosomes, to evidence Applicant's possession of the claimed subject matter as of the filing date of the instant application.

1) Description of Distinguishing Identifying Characteristics of Satellite Artificial Chromosomes

The instant application provides detailed identifying characteristics of satellite artificial chromosomes including detailed descriptions, with figures and schematics depicting the structure of artificial chromosomes. and working examples evidencing Applicant's possession of the claimed subject matter. For example, the application provides a detailed description of the common features of satellite artificial chromosomes that applies whether the satellite artificial chromosome is derived from an animal, plant or other cell. The application makes clear that the common attributes possessed by the members of the genus of satellite artificial chromosomes are relatively invariant: they have more heterochromatin than euchromatin and generally contain duplicated segments of DNA, which includes highly repetitive DNA, such as, for example, pericentric heterochromatic DNA or satellite DNA (see e.g., page 7, lines 15-20). Furthermore, all arise by the amplification events as described in the application. The application describes that, except for the heterologous nucleic acid such as a selectable marker or other foreign DNA, the SATACs contains only non-protein-encoding heterochromoatin (see page 7, lines 15-20). The application describes that this megachromosome (i.e. SATAC) is stable and can replicate and segregate alongside an endogenous chromosome (see, e.g., page 16, lines 22-35; page 34, line 23). The application describes how generate a satellite artificial chromosomes, how to identify a satellite artificial chromosome, for example, by C-banding and/or fluorescence in situ hybridization (FISH) using labeled nucleic acid probes (e.g., satellite DNA probes) to visualize heterochromatin and highly repetitive DNA. The application provides detailed descriptions of the structure of satellite artificial chromosomes, including the schematics in the figures. The instant application provides elaborate descriptions of an exemplary satellite artificial chromosome (i.e. a mouse megagchromosome) that was generated in a mouse cell line, which included C-banding studies revealing the primarily heterochromatic nature of satellite artificial chromosomes and FISH analyses showing that these satellite artificial chromosomes contain repeating units of satellite DNA. The same methods can be applied to any satellite artificial chromosome produced in any cell type.

The instant application also demonstrates possession of plant satellite artificial chromosomes as of the earliest filing date of the application. The specification describes that plant satellite artificial chromosomes are satellite artificial chromosomes that include plant centromeres (see for example, at page 16, lines 22-36). The specification describes exemplary structural elements of chromosomes, applicable to all species, which includes centromeres, telomeres, an origin of replication and heterochromatin (see, for example, page

7, lines 17-22; and also the section entitled "Identification and isolation of the components of artificial chromosomes." at pages 43-47 with subsections describing centromeres (page 44), telomeres (page 46), megareplicator (page 46), filler heterochromatin (page 46) and selectable markers (page 47). Hence, one of skill in the art could immediately envisage from the instant application a plant satellite artificial chromosome as a chromosome in the characteristic form of two arms extending from a constricted region of plant centromeric DNA, and containing more heterochromatin than euchromatin with interspersed heterologous DNA as depicted in the application.

The section of the specification entitled "Preparation of SATACs" at page 33, provides detailed descriptions of satellite artificial chromosomes and their characteristic structure. Cell lines containing satellite artificial chromosomes are described at page 36. Additionally, Example 6, pages 92, describes production of an exemplary satellite artificial chromosome. The application describes that this is an exemplary SATAC, and that such SATACs can be produced in other cells (see e.g., page 30, lines 9-12; and page 33, lines 20-22). The Example provides detailed description and characterization of the satellite artificial chromosome structure. Example 8 describes in situ studies of a megachromosome, an exemplary satellite artificial chromosome. The example characterizes replicating regions and features of the chromosomes. Example 9 describes the production of large scale amplification of pericentric regions in cells and analysis of the chromosomal products. Example 10 describes the purification of satellite artificial chromosomes using identifying characteristics such as atypical base content and size. The example describes the use of dyes such as Hoechst and chromomycin to stain and sort the chromosomes. Each of these examples provide detailed description and characterization of the satellite artificial chromosome structure. These detailed descriptions of satellite artificial chromosomes evidence Applicant's possession of the claimed subject mater.

Furthermore, the specification describes in great detail methods for generation of satellite artificial chromosomes. As described in the application, SATACs are generated following introduction of nucleic acid, such as heterologous nucleic acid encoding a selectable marker, into the heterochromatic pericentric region of the chromosome, whether by targeted introduction, for example, to promote homologous recombination or random introduction, which initiates amplification events leading to the generation of a de novo centromere, and ultimately a SATAC (see page 6, line 15 to page 7, line 24). Hence, a method includes the steps of introduction of DNA fragments into cells, which are then

cultured, identification of structures indicative of the requisite amplification event, and selection of cells that contain a satellite artificial chromosome or precursors thereof. As discussed above, the structures of these chromosomes are characterized and described in exquisite detail in the specification such that one of skill in the art could readily recognize and select cells containing them. The application describes that the methods of producing SATACs is applicable to the production of SATACs derived from any eukaryotic cells, for example, mammals, birds, fowl, fish, insects and plants (see page 9, lines 14-18).

The application describes how cells containing satellite artificial chromosomes are selected. For example, at page 8, line 23 to page 9, line 13 of the specification:

> **Also provided are SATACs of various sizes that are formed by repeated culturing under selective conditions and subcloning of cells that contain chromosomes produced from the formerly dicentric chromosomes.** The exemplified SATACs are based on repeating DNA units that are about 15 Mb (two ~7.5 Mb blocks). The repeating DNA unit of SATACs formed from other species and other chromosomes may vary, but typically would be on the order of about 7 to about 20 Mb. The repeating DNA units are referred to herein as megareplicons, which in the exemplified SATACs contain tandem blocks of satellite DNA flanked by non-satellite DNA, including heterologous DNA and non-satellite DNA. Amplification produces an array of chromosome segments [each called an amplicon] that contain two inverted megareplicons bordered by heterologous ["foreign"] DNA. **Repeated cell fusion, growth on selective medium and/or BrdU (5-bromodeoxyuridine] treatment or other treatment with other genome destabilizing reagent or agent, such as ionizing radiation, including X-rays, and subcloning results in cell lines that carry stable heterochromatic or partially heterochromatic chromosomes, including a 150-200 Mb "sausage" chromosome, a 500-1000 Mb gigachromosomes, a stable 250-400 Mb megachromosome and various smaller stable chromosomes derived therefrom.** These chromosomes are based on these repeating units and can include heterologous DNA that is expressed. (emphasis added)

Thus, satellite artificial chromosomes of various sizes are formed by repeated culturing under selective conditions and subcloning of cells that contain chromosomes produced from the formerly dicentric chromosomes. For example, the specification at page 34, lines 8-27 describes the production and identification of exemplary satellite artificial chromosomes including megachromosomes and gigachromosomes.

The specification provides a painstaking detailed analysis of each of the steps in a method of generating the satellite artificial chromosomes, characterizing each of the intermediate structures including dicentric/multicentric chromosomes, formerly dicentric chromosomes and sausage chromosomes in exquisite detail. The characterization of the

intermediates involved in the generation of the intermediates described in the specification provides an understanding of the mechanism underlying the method of satellite artificial chromosome formation. The specification describes that the process for generating satellite artificial chromosomes can be performed in any eukaryotic cell, including plants, and states that the methods and products are as described. Although the selection of cells containing satellite artificial chromosomes does not necessitate prior isolation and selection of cells containing precursor or intermediate species, the detailed description and characterization of the intermediate species in the specification further demonstrate that Applicant had possession of satellite artificial chromosomes at the time the application was filed, and that these chromosomes could be readily reproduced and identified. The Declarations of record demonstrate that, as described in the application, the plant chromosomes undergo the same fundamental amplification processes to generate the same sets of intermediates and ultimate product.

Hence, the description of the methods for generating satellite artificial chromosomes are applicant to any plant and animal species of satellite artificial chromosomes and any plant and animal cell type and can be used to generate transgenic plants therefrom. Although the working examples are exemplified in animal cells, the application explains that methods of generating satellite artificial chromosomes are applicable to any plant and animal cell type and any plant and animal species of satellite artificial chromosomes (see for example, at page 9, lines 15-19; page 30, lines 9-12). For example, at page 9, lines 15-24, the specification states:

> Thus, methods for producing MACs of both types (i.e. SATACs and minichromosomes) are provided. These methods are applicable to the production of artificial chromosomes containing centromeres derived from any higher eukaryotic cell, including mammals, birds, fowl, fish, insects and plants.
>
> The resulting chromosomes can be purified by methods provided herein to provide vectors for introduction of heterologous DNA into selected cells for production of the gene product(s) encoded by the heterologous DNA, for production of transgenic (non-human) animals, birds, fowl, fish and plants or for gene therapy.

2) Description of a Satellite Artificial Chromosome Provided as a Biological Deposit.

Additionally, the instant application provides exemplary SATACs evidencing Applicant's possession of the claimed subject matter. For example, the specification describes the generation of cell lines such as G3D5 and H1D3 and mM2C1. Each of these

cell lines contains a satellite artificial chromosome. Further, the satellite artificial chromosomes contained in the cell lines are described and characterized in detail (see for example, pages 36-39 and Example 6). Moreover, while not necessary for purposes of 35 U.S.C. §112, first paragraph, G3D5 and H1D3 cell lines, which contain satellite artificial chromosomes, have been deposited with the ECACC under accession nos. 96040928 and 96040929, respectively (page 74, line 22 to page 75, line 7). The specification makes it clear that the deposited satellite artificial chromosomes are exemplary. Such deposition is unequivocal demonstration of possession of satellite artificial chromosomes.

3) Depiction of a Satellite Artificial Chromosome in Detailed Drawing

Additionally, Applicant's possession of the claimed subject matter is demonstrated by the detailed figures depicting the structures of satellite artificial chromosomes. For example, Figures 2 and 3 depict the formation of satellite artificial chromosomes, including a megachromosome and intermediates. The specification depicts the structures of SATACs schematically in Figures 2 and 3. In particular, formation of a satellite artificial chromosomes shown in Figures 2D, 2E, and 2F and an exemplary satellite artificial chromosome structure is depicted in Figures 2F and 3. These drawings were based on detailed studies, described in the application, of the satellite artificial chromosomes, including *in situ* hybridization with satellite DNA (as a probe) for heterochromatic DNA, euchromatic probes and Hoechst staining and FPG staining to observe chromosome architecture within a satellite artificial chromosome. In addition, pulse field gel electrophoresis and southern hybridization were used to map satellite artificial chromosomes. Cloning and sequencing of regions of satellite artificial chromosomes were employed to confirm the structure of the chromosomes. Additionally, the satellite artificial chromosomes were observed directly by scanning electron microscopy (see, for example, pages 93-103 describing a detailed analysis of a megachromosome, an example of a satellite artificial chromosome).

The Detailed Description, Working Examples, Biological Deposit and Drawings of SATACs and their Identifying Characteristics Proves Possession of a Genus of SATACs in Compliance with the Written Description Requirement

As explained above, the description of satellite artificial chromosomes in the instant application demonstrates possession of the genus through **all** of the exemplary ways cited in the Guidelines for Examination under 35 U.S.C. §112, first paragraph. As discussed in the previous response dated November 09, 2005, the Guidelines for Examination under 35 U.S.C. §112, first paragraph also sets forth that a description of an actual reduction to

practice of a particular species is but one of many ways to satisfy the written description requirement and is by no means necessary to satisfy the requirement. See, *e.g., Pfaff v. Wells Electronics, Inc.*, 55 U.S. at 66, 119 S.Ct. at 311, 48 USPQ2d at 1646 (1998) ("...just because reduction to practice is sufficient evidence of completion, it dos not follow that proof of reduction to practice is necessary in every case. Indeed,...one can prove that an invention is complete and ready for patenting before it has actually been reduced to practice.") Additional ways to demonstrate satisfaction of the written description requirement with respect to a particular species include a description of the claimed subject matter in terms of distinguishing identifying characteristics as evidenced by other descriptions of the invention that are sufficiently detailed to show that applicant was in possession of the claimed subject matter. Factors to consider include (1) the level of skill and knowledge in the art, (2) partial structure, (3) physical and chemical properties, (4) the functional characteristics alone or coupled with a known or disclosed correlation between structure and function, and (6) the methods of making the claimed subject matter. Furthermore, the DECLARATIONs provide confirmatory evidence to rebut any statement by the Office that plant satellite artificial chromosomes would have a different structure or would not be generated as described in the application.

In view of the abundance of information provided in connection with the description of the distinguishing and identifying characteristics of satellite artificial chromosomes, it is essentially unimaginable that a person skilled in the art would have any doubt whatsoever that the inventor had possession of satellite artificial chromosomes. Not only does the application demonstrate possession in **all** of the exemplary ways suggested by the Guideline for Examination under 35 U.S.C. §112, first paragraph (based on established law), an assessment of the factors to be considered in determining whether there is sufficient evidence of possession of claimed subject matter also leads to the clear conclusion that the application satisfies the requirements of 35 U.S.C. §112, first paragraph. Among these factors are: (1) the level of skill and knowledge in the art is high, (2) the full and distinctive structure of a satellite artificial chromosome (including two arms containing extensively duplicated DNA segments, and a centromeric constriction) is described and depicted in drawings, (3) the distinguishing physical properties, such as the primarily heterochromatic nature of the artificial chromosomes, are elaborated, (4) the chemical properties of DNA in general are well-known in the art, (5) the functional characteristics of satellite artificial chromosomes (e.g., replication, segregation and maintenance within cells, high-level expression of coding

sequences contain within) which are correlated to its structure as a chromosome are demonstrated through actual experimental results, and (6) methods of making satellite artificial chromosomes are outline in step-by-step detail. Applicant respectfully urges that the application and figures be reviewed as a whole, and questions how such an elaborate description, including description of the universal identifying features of SATACs and methods of making SATACs, figures, and description of actual reduction to practice satellite artificial chromosomes, could possibly fail to demonstrate possession of a genus of SATACs.

The written description requirement can be satisfied without express or explicit disclosure of the claimed subject matter. See e.g, In re Herschler, 591 F. 2d 693, 700, 200 USPQ 711, 717 (CCPA 1979); Purdue Pharma L.P. v Faulding, Inc. 230 F.3d 1320, 1323, 56 USPQ 2d 1481, 1483 (Fed. Cir. 2000). Hence, the application need not provide representative species of every type of satellite artificial chromosomes, if the descriptions provided therein are descriptive of all satellite artificial chromosomes. As noted above, the application indicates the characteristics of satellite artificial chromosomes described therein are applicable to all eukaryotic satellite artificial chromosomes. The specification explicitly states that plant satellite artificial chromosomes can be made by the methods therein. Thus, the detailed descriptions of structural and functional characteristics of satellite artificial chromosomes are directly applicable to plant satellite artificial chromosomes and other eukaryotic species. Moreover, the satellite artificial chromosomes and cells containing satellite artificial chromosomes provided in the specification are representative of plant satellite artificial chromosomes, since, as described, the identifying characteristics are common to all satellite artificial chromosomes.

Applicant respectfully submits that the Examiner has not provided any basis to doubt the veracity of this assertion. The application sets forth identifying characteristics that are not limited by species. The Examiner has provided no substantiated reasons why such identifying features are not common to all satellite artificial chromosomes, nor why such features are not sufficient to identify and show possession of plant satellite artificial chromosomes. As noted above, the Applicant need not provide a representative of everything claimed, but may show possession by providing identifying features common to all members. Applicant, by way of detailed descriptions of features, figures, working examples and exemplary satellite artificial chromosomes and cell lines, has done exactly that. Such disclosures evidence Applicant's possession of plant satellite artificial chromosomes and cells

containing such chromosomes. Therefore, it is respectfully submitted that Applicant had possession of the claimed subject matter as the time the instant application was filed.

CONCLUSION

In summary, the detailed descriptions, figures and cell lines all demonstrate Applicant's possession of satellite artificial chromosomes as of the filing of the application. Moreover, these figures and accompanying descriptions are disclosed in the parent applications, U.S. application serial nos. 08/695,191, 08/692,080 and 08/629,822, which are incorporated in their entirety by reference into the instant application, evidencing Applicant's possession of satellite artificial chromosomes as of the earliest filing date of the application.

The primary purpose of the written description requirement is to demonstrate that the Applicant was in possession of the invention at the time of the filing date sought. Vas-Cath, Inc. v. Mahurkar, 935 F.2d 1555, 1563-64, 19 USPQ.2d 1111, 1117 (Fed. Cir. 1991). As addressed above, the instant application provides sufficient description of relevant identifying characteristics of SATACs and plant SATACs to evidence Applicant's possession of the claimed subject matter at the time of filing.

DECLARATIONS

Furthermore, in the instant case, the description of satellite artificial chromosomes provided in the instant application identifies the actual physical embodiments of any satellite artificial chromosome, including any animal or plant satellite artificial chromosome, generated using methods described in the application (see methods and results provided in Fabijanski Declaration 2 filed April 22, 2204 and Fabijanski Declaration 4 filed November 09, 2005, and the Declarations 5 and 6 provided herewith). Based on the description and teachings of the application, the application and Declarations evidence that SATACs can be identified from mouse, humans and diverse plant species based on the presence of a distinct chromosome containing predominantly heterochromatic arms and a centromere using techniques such as C-banding, in situ hybridization (FISH) and Southern hybridization. Hence, the Declarations show that, as described in the application, SATACs, and precursors and intermediates of SATACs (i.e. dicentric chromosomes, sausage chromosomes and gigachromosomes,) contain common identifying characteristics.

Declarations 2, 4 and 5 – Plant SATACs

Previous declarations of record (see Declaration 2 and Declaration 4) evidence that the description of SATACs in the instant application identifies the actual physical embodiments of a plant satellite artificial chromosome generated using methods described in

the application. These Declarations show results of the generation of plant SATACs obtained by PEG-mediated transfection of tobacco protoplasts with heterologous DNA. Using fluorescence in situ hybridization (FISH) for visualization of pericentric heterochromatin and the heterologous DNA, based on the description of a satellite artificial chromosome in the application, a plant satellite artificial chromosomes were identified . For example, as set forth in Fabijanksi Declaration 2, a plant satellite artificial chromosome was clearly visible in chromosome spreads as a small independent chromosome entity containing amplified heterologous DNA and pericentric DNA. Metaphase images of the plant SATAC demonstrated the presence of small chromosome arms and a constriction representing the centromere region containing plant centromeric DNA. Figure 2 (A) and (C) of Declaration 4 show the results of DAPI-stained chromosomes co-stained using a FITC-labeled probe specific for a portion of the heterologous DNA (i.e. a mouse major satellite DNA sequence) and a rhodamine red-labeled probe specific for tobacco pericentric heterochromatin (i.e., 18S rDNA). The results reveal that the entire plant SATAC chromosome hybridized to the 18S rDNA-specific probe. Also, significant hybridization of the heterologous DNA-specific probe to the plant SATAC reveals the co-amplified heterologous DNA contained within the SATAC. Thus, as described in the instant application, the plant SATAC generated as described in the application, is predominantly heterochromatin (i.e. rDNA) with interspersed heterologous DNA. This is particularly evident in an overlay of the FITC-labeled and rhodamine-labeled image analyses of the plant SATAC (see Figure 3 of Fabijanski Declaration 4). In addition, the callus line containing the plant SATAC was stably maintained in culture for well over six months (see Fabijanski Declaration 2), thereby demonstrating the correlation between the function (replication and stable maintenance in cells) and structure of the plant SATAC as described in the instant application.

In addition, to further evidence that the method of generating plant SATACs is applicable to diverse species of plants, attached is a Declaration 5 under 37 C.F.R. §1.132 of Steven F. Fabijanski. The Declaration details construction of plant SATACs in two distinct plant species, *Nicotiana* and *Brassica*. Declaration 5 shows the generation of plant SATACs from both plant species having the same identifying characteristics of SATACs as described in the instant application. The plant SATAC contains a plant centromere, as well as amplified pericentric DNA and the introduced heterologous DNA *just as described in the application as originally filed* (including the ultimate parent application).

As described in the instant application, SATACs were generated following introduction of heterologous DNA encoding a selectable marker, either phosphinothricin N-acetyltransferase (PAT) gene or phosphinothricin acetyl transferase gene (*bar)*, into *Nicotiana* protoplasts or *Brassica napus* protoplasts, respectively. While not necessary, the DNA fragment also included rDNA to target it to the pericentric region of plant chromosomes. Following growth under selective conditions and selection of cells, two-color fluorescence in situ hybridization (FISH) was used to co-stain metaphase chromosomes for the selectable marker (yellow-green signal) and for pericentric heterochromatin 18s rDNA (red signal). As described in the application, Declaration 5 shows that the DNA fragment integrated into the plant chromosome necessary leading to the amplification event, which was then exploited to generate a SATAC in plant cells. For example, the Declaration 5 shows that the amplified selectable marker sequences are within amplified pericentric DNA evidencing integration into the heterochromatic pericentric region. Also, the Declaration 5 describes that areas where significant levels of both yellow-green and red signals were observed demonstrate large-scale amplification of the pericentric DNA. Using the in situ hybridization as described in the application, the Declaration 5 depicts the identifying characteristics of precursor and intermediates in the formation of SATACs (i.e. dicentric chromosomes and sausage chromosomes), and the identifying characteristics of SATACs, as described in the application. Thus, following the methods described in the instant application, SATACs were generated and identified in *Nicotiana* and *Brassica* plant species. They were identified by the characteristics described in the application, *i.e.*, by the presence of a substantial amount of amplified pericentric heterochromatic DNA and a selectable marker on a distinct chromosome structure. As set forth in the Declaration 5, no knowledge of the plant centromere sequence nor any sequence was required to generate or identify a SATAC, including a plant SATAC.

Clearly, the description of a SATACs, including plant SATACs, provided in the instant application describes the plant SATACs depicted in each of the accompanying Declarations of Fabijanksi (Declaration 2, Declaration 4 and Declaration 5). Thus, there can be no doubt that plant SATACs are sufficiently described in the instant application to demonstrate Applicant's possession of these artificial chromosomes.

Declaration 6 – Methods of Generating and Identifying Characteristics of SATACs are Universal

As discussed above, a DECLARATION 6 under 37 C.F.R. § 1.132 of Dr. Gyula Hadlaczky, an inventor of the claimed subject matter, is provided herewith. The Declaration 6 is provided to show the pioneering nature of the discovery of the amplification events and the universality of the underlying chromosomal processes involved in the generation of the dicentric chromosomes and ultimately SATACs and of the process for production of SATACs described in the application. Declaration 6 demonstrates that as described in the application, SATACs from diverse species, such as plants and mammals, have been prepared by the methods taught in the specification, and share the same identifying characteristics. The Declaration 6 demonstrates that the methodology generically described in the application and exemplified with rodent chromosomes, is reproducible. Further, the Declaration 6 points out that the methods disclosed in the application are based on universal amplification events common to all eukaryotic chromosomes. Based on data provided in Declaration 6 and the accompanying Declaration 5 of Fabijanski, the universality is evidenced by the fact that SATACs can be prepared in accord with the teachings of the application in species as diverse as mammalian species and plant species. Surely, if the amplification occurs in plants, humans and rodents, it is can be inferred that it is a universal phenomena that occurs in other mammals, including whales and gorillas and dolphins and rats and apes.

Rebuttal to Examiner's Arguments

1) No knowledge of a centromeric sequence is required to generate, identify or use a SATAC

The Examiner states that Applicant's contention that plant SATACs do not have any particular structural requirements is contradicted by Applicant's specification, which states that "plant artificial chromosomes... refer to chromosomes that include...plant centromeres." It is respectfully submitted that Applicant did not state that a plant SATAC does not have any particular structural chromosomes as alleged by the Examiner. As described in detail in the application and in previous responses, a SATAC is a chromosome and thus has many of the same structural features of a chromosome, including a centromere. Hence, as described in the application, a plant SATAC has a plant centromere. A SATAC, including a plant SATAC, is distinguished from a "wildtype" chromosome because it contains arrays of repeating units of duplicated DNA and more heterochromatin than euchromatin. As discussed in great detail above, the application describes and depicts the structural features of satellite artificial chromosomes and teaches how to generate them, identify and isolate them.

This is described in the application, and has exhaustively been detailed in the previous responses and above.

Notwithstanding this, as discussed above, **the sequence of the centromere or knowledge of the centromere is not required to generate, identify or use a SATAC.** Centromeric DNA sequence information is not required for the generation of a satellite artificial chromosome in any eukaryotic cell, including plants. Cells, when treated as described in the instant application, generate chromosome structures from which satellite artificial chromosomes can be produced.

Hence, the Examiner's assertion that it is unclear whether plant and animal chromosomes and centromeres have appreciable sequence conservation or any conserved domains is inapt. The conservation in sequence of centromeres between animals, plants and other species is not relevant to the generation, identification and use of SATACs. Thus, the reference of Jiang et al. (Trends in Plant Science, 8:570-575 (2003)) is not relevant to the generation of SATACs in diverse species. As described in detail above, and as exemplified in the application and in the Declarations of record and those provided herewith, SATACs can be generated in diverse species, including mouse, human and plants, by introducing heterologous DNA into a cell, growing the cells, and ultimately identifying those cells that contain SATACs based on identifying characteristics such as the presence of primarily pericentric heterochromatic DNA. Such chromosomes also have centromeres, but knowledge of the centromeric sequences is not necessary to generate or identify SATACs. For example, the Declaration 5 of Fabijanski describes that there is no centromere specific probe for Nicotiana, yet SATACs were identified in Nicotiana based on in situ hybridization FISH analysis for the presence of pericentric heterochromatic DNA and the selectable marker.

2) Plant SATACs are described in the instant application, and are further evidenced in Declarations 2, 4 and 5

The Examiner urges that the case for a plant satellite artificial chromosome is not persuasive because as stated in the Office Action mailed 22 October 2003, "the declaration is lacking any teaching of [a method of producing] a plant SATAC, a plant SATAC in a plant cell, a plant SATAC in a plant, or a plant SATAC in an animal cell." The Examiner goes on to say that the Declaration of Fabijanski fails to provide any identifiable components of a plant SATAC as claimed. The Examiner also urges that Declaration 1 lacks any teaching of a plant SATAC or any identifiable components of a plant SATAC. The Examiner states that the Declaration provides no location of the satellite artificial chromosome, the size of any

remaining nucleic acid sequence is unspecified, and no information is provided regarding the possible function of integration sites of such nucleic acids. This is incorrect.

It appears that the Examiner is referring to Declaration 1 submitted in the response mailed July 16, 2003. It is respectfully submitted that indeed Declaration 1 did not describe a plant SATAC. As described in the response mailed April 22, 2004 (see page 54-56), Applicant already has rebutted each of the points raised in the Examiner's statement above in the Office Action mailed October 22, 2003. Declaration 1 demonstrates the operability of methods of introducing SATACs into cells, including SATACs of one species into cells of another species, and identifying the SATACs. The SATAC exemplified in Declaration 1 was a mammalian SATAC and was introduced into tobacco protoplast cells, Arabidopsis cells and rice protoplasts, evidencing that one of skill in the art can introduce a mammalian satellite artificial chromosome into a plant cell (to address a rejection of record at that time).

Although Declaration 1 does not describe a plant SATAC, it is respectfully submitted that Applicant has demonstrated generation of a plant SATAC and a plant SATAC in a plant cell. For example, as detailed above, each of Declarations 2, 4, and 5 demonstrate the generation and characterization of plant SATACs based on identifying characteristics common to all SATACs as described in the application. As described in the application, Declaration 6 of Hadlaczky describes that the universal process of generating SATACs in diverse species. This is substantiated by each of Declarations 2, 4, 5 and 6 and the application, which describe that SATACs, regardless of the species from which they are produced, are generated and can be identified based on identifying characteristics as described in the application, i.e. repeating units of DNA and primarily pericentric heterochromatin. Hence, Applicant has demonstrated plant SATACs. As discussed in detail above, the application describes in exquisite detail the identifying characteristics of SATACs, which is universal to all SATACs, including plants.

In addition, Applicant does not see the relevance about the comments regarding the size of any remaining nucleic acid or the function of integration sites of such nucleic acids. It is unclear to which nucleic acids the Examiner is referring; but it is unequivocal that such information or knowledge is of no relevance. The Declaration 1 demonstrates introduction of SATACs into tobacco cells, Arabidopsis cells, and rice cells. As described in the application and in the Declaration, the SATACs are maintained stably in cells. The SATACs are not genomically integrated. Declaration 1 describes that the SATACs were detected in plant cells for at least 16 weeks. Given this evidence, there is no clear basis or reasons given that

would support the Examiner's assertions regarding the results set forth in the Declaration of Fabijanski, nor any reason to believe that a SATAC was not generated and stably maintained as described by Dr. Fabijanski. Notwithstanding this, the Declaration 1 supports the claimed subject matter by demonstrating the introduction of SATACs into diverse plant cells, including tobacco, Arabidopsis and rice.

3) The Depiction of the Identifying Characteristics of SATACs Described in Figures 2 and 3 correlates with Figures 1, 2 and 3 of Declaration 4

The Examiner urges that the relationship of the specifics of the specification figures 2 and 3 with those of the Fabijanski figures 1, 2, and 3 [in Declaration 4] is not apparent. The Examiner states that the Figures in Declaration 4 cannot be evaluated until a detailed correlation, which includes the indicated identified area of the various panels of the figures, is provided, including what are the "sausage chromosomes," "stable megachromosomes," and "gigachromosomes."

It is respectfully submitted that Figures 2 and 3 in the specification are model depictions of the generation of SATACs and the identifying characteristics of such SATACs. In contrast, Figures 1, 2, and 3 in the Declaration are actual pictures of SATAC structures as assessed by FISH analysis. It is not surprising, therefore, that the Figures are not identical in appearance; a schematic and photograph are necessarily different. Figures 2 and 3 in the application depict common identifying features of SATACs; the analyses of SATACs in Figures 1, 2 and 3 in Declaration 4 are based on the identifying characteristics of SATACs using methods as described in detail in the application, including the Working Examples, *i.e.* in situ hybridization. Thus, the analysis of the results should take into consideration the detailed description, identifying features of SATACs, and methods of identifying such features as described in the application as a whole. As noted above, the Figures are only one way that evidence the description and possession of SATACs.

Nevertheless, the features depicted in the SATACs in Figures 2 and 3 do correlate with the depiction of the identifying characteristics of SATACs depicted in Figures 1, 2 and 3 of Declaration 4. Figure 2 of the application depicts the process of SATAC generation as described in the application (see e.g., page 7, line 12 to page 8, line 13; page 33, line 19 to page 34, line 27), ultimately leading to the generation of a megachromosome (i.e. SATAC; panel 2-8 of Figure 2). For example, Figure 2 of the application depicts the introduction of heterologous DNA into a cell by targeted integration to the pericentric region (panel 2-1) resulting in amplification of the heterochromatic region and generation of a de novo

centromere (i.e. neo-centromere) and the formation of a minichromosome and a formerly dicentric chromosome (see 2B, i.e. panel 2-2 and 2-3). Also, as described in the application, repeated culturing of the cells containing the dicentric chromosome results in chromosome structures such as a sausage chromosome (see 2D, i.e. panel 2-5), gigachromosome (see 2E, i.e. panel 2-7) and megachromosome (i.e. SATAC; see 2F, i.e. panel 2-8). Each of these structures can be identified and selected.

As discussed in detail in the Declaration and above, the plant SATAC described in Declaration 4 was generated based on the method as described in the application and in Figure 2 by introducing heterologous DNA along with a targeting sequence to target the foreign DNA to the pericentric region, which initiated amplification events resulting in the formation of a de novo chromosome and ultimately the generation of a SATAC and other chromosome intermediates (i.e. sausage chromosomes). Using FISH analysis, these structures were identified as set forth in Figures 1, 2, and 3. For example, both Figure 2 in Declaration 4 has arrows drawn to indicate the SATAC structure and the sausage chromosome structure (SAR). Although each of these structures were identified based on identifying characteristics as described in the application and in Figure 2, it is respectfully submitted that the claimed subject matter is directed to a SATAC. Therefore, although the Declaration 4 did provide a picture of a sausage chromosome structure, this is not relevant to the claimed subject matter. Likewise, it is not relevant that the Figures in Declaration 4 do not depict a gigachromosome. Although Dr. Fabijanski could likely provide numerous pictures of gigachromosomes and other intermediates, the Declaration 4 demonstrates generation of SATACs in plants.

Hence, the most appropriate correlating portion of either Figures 2 or 3 is the bottom panel of each, i.e. Figure 2F (panel 2-8), and the bottom structure depicted in Figure 3, which each depict the identifying characteristics of a SATAC. The bottom panels of each of Figures 2 and 3 depict a distinct chromosome structure that is predominately heterochromatin. It also depicts a structure that is interspersed with foreign DNA that has been integrated into the de novo formed chromosome based on the homologous recombination and amplification events that occur during the formation of the SATAC. The structure shows the presence of two heterochromatic arms, and a centromere.

Declaration 4 depicts these identifying features in Figures 1, 2 and 3 based on the results of FISH analysis. Spreads of chromosomes were stained with DAPI to identify chromosome structure, including wild-type chromosomes. The chromosome spreads were

then co-stained with a marker-specific probe to detect the heterologous foreign DNA (which is the blue/green signal in the Figures 1, 2 and 3 in the Declaration 4, and with a probe that recognizes the pericentric tobacco 18s rDNA (which is the red signal). For example, Panel A of Figure 1 in Declaration 4 shows hybridization of the vector DNA probe to chromosome spreads. As described in the application and in the Declaration, SATACs are formed following large-scale amplification of the heterologous DNA, which in this instance in Panel A is evidenced by the high intensity blue/green signal (as compared to other chromosome spreads not depicted that had low levels of blue/green intensity.) Panel B of Figure 1 in Declaration 4 depicts hybridization of the 18srDNA probe. Because 18s rDNA is present in all tobacco chromosomes, there is a low level red staining of all chromosomes, however, the intensity of the red stain signifies a large-scale amplification of the pericentric DNA and a chromosome structure that is predominantly heterochromatin. Also, the portion of Figure 1 that is circled evidences a distinct chromosome structure that is predominantly hetero-chromatic and contains heterologous DNA. This is a SATAC.

This SATAC is depicted in more detail in Figure 2 in Declaration 4, which shows a higher magnification image of this area (Panel A: DAPI staining for the chromosome; Panel B: blue/green signal evidencing hybridization of the heterologous marker probe; Panel C: red signal evidencing hybridization of the 18S rDNA probe). Figure 3 of Declaration 4 depicts an overlay of each of these hybridization signals from two independent chromosome spreads as set forth in Panel A and Panel B. In high-resolution images, the presence of both amplified vector (blue/green signal) and pericentric heterochromatic DNA (red signal) was observed co-localized to a single chromosome structure. Also, the images depict the presence of two heterochromatin arms and a constriction representing the centromere region.

These results as depicted in Figures 1, 2, and 3 in Declaration 4 correlate with the structure of a SATAC, including all identifying characteristics, as described in the bottom panel of Figures 2 and 3 in the application. Hence, the Declaration 4 evidences possession of a plant SATAC as described in the application.

4) A SATAC, including a plant SATAC, can be identified by the presence of an amplification of pericentric DNA (rDNA) in an independent chromosome structure

The Examiner states that the Declaration 4 does not provide any sequence information regarding which tobacco sequences were conserved in the SATAC produced from the tobacco genomic sequences. The Examiner urges that the Applicant has not provided any

structural features of a second species of the claimed genus, which would be required to demonstrate the conservation of sequence between the two species, namely mouse SATAC and tobacco SATAC.

It is respectfully submitted that there is no need to provide sequence information nor conservation of sequence. Satellite artificial chromosomes are generated by amplification events that occur upon integration of a DNA fragment in the heterochromatic pericentric region of a chromosome. It is the gross structural features that are key, not the particular sequences. The application teaches how to generate a SATAC and the structural features by which it can be identified. The application provides exhaustive structural detail and identifying characteristics of a SATAC, including a plant SATAC. As described in detail in this and the previous response, the plant SATAC in Declaration 4 (and Declaration 2 and Declaration 5 provided herewith) contain all the identifying characteristics of a SATAC as described in the application, including the characteristic structural features of arms and a centromeric region that can be identified and distinguished on the basis of the predominance of heterochromatin in the chromosome. These identifying characteristics are described in detail throughout the Declaration, and are depicted in Figures 1, 2 and 3.

Also, contrary to the assertions by the Examiner, the Figures in Declaration 4 do depict the tobacco sequences present in the SATAC, i.e. the pericentric heterochromatic sequences. This is shown by in situ hybridization using a rhodamine-tagged probe (red fluorescence) and recognized pericentric DNA (18S rDNA) sequences endogenous to tobacco cells (see page 5, lines 19-20) of Declaration 4. Hence, Applicant has shown the presence of tobacco heterochromatic sequences in the SATACs. Thus, while not necessary, sequence information of the SATAC is known based on its hybridization to the 18s rDNA probe.

5) The Declaration 4, and other Declarations of record, evidence the generation of SATACs, including plant SATACs, based on the description in the specification

The Examiner acknowledges that Declaration 4 demonstrates the generation of plant SATACs. The Examiner, however, continues to maintain that the use of a tobacco genomic sequence submitted in 1996 does not comply with the Written Description Guidelines, which mandate that the specification provide adequate written description as of its own filing date.

First, it is respectfully submitted that the comment by the Examiner acknowledging the generation of a SATAC in Declaration 4 seemingly contradicts the Examiner's earlier statement that there is no teaching of a plant SATAC (see point 2) above). Again, it is noted,

and as acknowledged by the Examiner, that Declaration 4 indeed does demonstrate the generation of a plant SATAC based on the description in the application.

Second, as described in detail above, Applicant most definitely has complied with the written description guidelines and adequately described SATACs and their generation as of the earliest filing date. As described in the earliest filed application filed April 10, 1996, to generate a SATAC it only is necessary to introduce a piece of heterologous DNA into a cell, grow the cell, and look for cells that contain formerly dicentric chromosomes from which satellite artificial chromosomes can be generated. Use of a selectable marker on the piece of heterologous DNA renders it easier to find the cells in which the events that lead to the generation of satellite artificial chromosomes occur. Such cells can them be selected, and a satellite artificial chromosome isolated therefrom. Also, as described in the earliest filed application, the heterologous DNA can be introduced with a sequence that targets it to the pericentric region of the chromosome, although even without a targeting sequence some heterologous DNA will be integrated into the pericentric region at some frequency. The application, as of its earliest filing date, evidences the identifying characteristics of SATACs. The heterologous DNA from 1996 is an APS sequence that is not part of generation of a SATAC as described in the application; it is just heterologous DNA. As described in the Declaration the DNA fragment that was introduced includes rDNA for targeting (optional as described in the application) and a selectable marker. The 1996 sequence could have been any nucleic acid molecule. (To prove this a new Declaration is submitted herewith).

Furthermore, as discussed in detail above, Declaration 4, and the other Declarations of record, provide evidence of 1) introduction of SATACs into cells (Declaration 1), 2) generation of plant SATACs (Declarations 2, 4 and 5) and 3) production of transgenic plants by introducing a SATAC into a plant cell (Declaration 3) **based on the description in the specification**. For example, Declaration 4 describes the generation of a SATAC by introducing DNA encoding a selectable marker (i.e. hygromycin phosphotransferase (HPT) and phosphinothricin acetyl transferase (PAT)) and, the albeit optional, DNA encoding a targeting sequence (i.e. Arabidopsis rDNA). The Declaration 4 shows the generation of a plant SATAC. Hence, Declaration 4 evidences the generation of a plant SATAC based on the description in the specification as of the earliest filing date, which teaches that one has to introduce DNA and look for amplification event.

Although Declaration 4 also describes other components that are introduced (i.e. the 334 bp tobacco pericentric sequence). For example, the 334 bp tobacco sequence is an

amplification promoting sequence. It is not required. The Declaration 5 of Fabijanski provided herewith evidences this because plant SATACs were generated by introducing heterologous DNA and into two different species of plant cells. The 334 bp tobacco pericentric sequence was not included in the heterologous DNA. Thus, the fact that this sequence **may** have been deposited in 1996 after the earliest filing date is irrelevant. Plant SATACs can be generated without this sequence based on the description in the application at of its earliest filing date.

6) Issued Patents 6,025,155 and 6,077,697 are presumed valid, and the term "satellite artificial chromosome" is recited in the issued claims, such that the description of satellite artificial chromosomes in the patents is presumptively adequate to satisfy the requirements of 35 U.S.C. §112, first paragraph

Responsive to Applicant's arguments regarding the presumed validity of patented claims in the parent patents, issued as 6,025,155 and 6,077,697, the Examiner states that because the Written Description Guidelines were not published in the Federal Gazette until January 2001, the instant Office Action contains a written description rejection not applied in the allowed parent applications.

It is respectfully submitted that the Written Description Guidelines are not the law, but are merely an interpretation of the law by the United States Patent Office. The Written Description Guidelines are not adequate legal authority by which the Office is permitted to disregard the validity of a U.S. Patent and the subject matter claimed. The Office is reminded that 35 U.S.C. §282 states that an issued patent is presumed valid. As discussed above, to ignore this presumption is to undermine the integrity of the US Patent system and those who rely upon this presumption.

III. REJECTION OF CLAIMS 50-52 and 73-128 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 50-52 and 73-128 are rejected under 35 U.S.C. §112, first paragraph in part for reasons of record as set forth in the Office Actions mailed October 22, 2003 and for reasons of record as set forth in the Office Actions mailed May 9, 2005. Specifically, the Examiner urges that the claims broadly read on any transformed plant of any of a multitude of unrelated species; and since the claims read on a multitude of recalcitrant monocotyledonous species, the specification does not provide any teaching of plant transformation of any species, which would be required to overcome the evidence of unpredictability inherent in obtaining transformed plant cells as clamed [as evidenced by Ohgawara et al. (1983) and Portykus (1990)]. The Examiner also alleges that Applicant has

only provided guidance for a single species of SATAC, namely a mouse SATAC, and undue experimentation would have been required to develop a multitude of SATACs from a multitude of non-exemplified animal and plant species, and to evaluate the persistence of these SATACs in plant cells following lipid-mediated transfection and other means.

The Examiner finds the response mailed November 09, 2005, unpersuasive for various reasons as follows: 1) it is unclear whether "cationic lipid-mediated transfection" as utilized in the prior Fabijanski declarations is the same as "lipid-mediated transfection" as instantly claimed and whether the instant specification provides support for either; 2) the rapid degradation of foreign DNA molecules reported by Ohgawara *et al.* would be a concern; 3) that "direct DNA transfer" is the "most efficient" technique; 4) that the specification is silent with regard to monocot transformation or the particularly claimed transformation methods; and 4) that the Declaration 4 of Fabijanski is unpersuasive because it is not supported by the specification as of the filing date. In addition, the Examiner cites Genentech, Inc. v. Novo Nordisk, A/S, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997) and Bayer v. Housey, 68 USPQ2d 1001, 1008-1009 (Fed. Cir. 2003) to evidence the lack of enablement of the instantly claimed methods. The rejection is respectfully traversed.

Summary of arguments below:

1) For reasons of record as provided in previous responses (incorporated by reference herein), Applicant respectfully submits that the specification is enabled generally to SATACs from any species, including mouse, human and plants. The Declarations 2 and 4 of record evidence the generation of plant SATACs based on the teachings of the specification. As detailed below, the fact that the Fabijanski Declaration 4 (which is a more detailed version of Declaration 2, including Figures) introduces heterologous nucleic acid whose sequence **may** have only been available after the earliest effective filing date is not relevant, and does not negate the fact that each of the Declarations of record are based on the teachings of the specification as of the earliest filing date.

In addition, although not needed, and as discussed above with respect to the Written Description rejection, a Declaration 5 under 37 C.F.R. §1.132 of Fabijanski, is provided, which further demonstrates the generation of plant SATACs in **diverse** species of plants, including monocots, using methods as described in the instant application and priority applications. This data evidence the universality of the fundamental process involved in the generation of SATACs, and also belies all of the comments of the Examiner regarding why the process would not operate. Also provided is a Declaration 6 under 37 C.F.R. §1.132 of

Hadlaczky, an inventor, demonstrating that the methods described in the application and the priority applications are based on fundamental chromosomal processes and are universally applicable to generation of SATACs, from any eukaryotic chromosome, including animals and plants. The instant Applicant is the first to identify such fundamental and universal events and their use to generate satellite artificial chromosomes. As such, this is a pioneering discovery entitled to broad scope. As noted in the Declaration 6, Dr. Hadlaczky is the recipient of a prestigious award in Hungary recognizing the pioneering nature of his discovery.

2) Applicant need not teach that which is well known. Furthermore, the standard for enablement is **NOT** that ***only*** the method that works best is enabling. There is no requirement that all methods have to work well, just that they have to work such that one of skill in the art, without undue experimentation, can practice the method as claimed. Also, some degree of inoperativeness is permitted. In this instance, the application not only teaches numerous methods of plant transformation, but the Declarations of record also evidence such transformation **in monocot and dicot plant species** based on the teachings in the specification. The Examiner must give deference to the teachings of the specification and to the Declarations, unless they can show that one of skill in the art would have a rational basis to doubt the truth of such statements

3) The Examiner's reliance on Ohgawara *et al.* (1983) and Potrykus (1990) is misplaced. First, these references are old (the Ohgawara *et al.* reference was published **13 years** before the effective filing date of the instant application), and do not evidence the state of the art of the application as filed. Second, the claims are directed to the introduction of SATACs into cells, **not** DNA or plasmid DNA. As detailed in the previous response filed November 19, 2005, and as taught in the application, SATACs are **stable**, replicating chromosomal vectors that overcome the limitations of plasmid DNA. The Declarations of record support this based on the detection of SATACs in cells for months after transformation. Accordingly, the Examiner's basis for questioning the maintenance of SATACs in cells upon transformation (i.e., due to rapid degradation of foreign DNA molecules) by analogy to the data described in Ohgawara *et al.* and Potrykus is inapt, and again does not give deference to statements made in the specification and in Declarations of record.

4) It is respectfully submitted that Applicant has addressed in great detail in numerous previous responses (each incorporated by reference herein) that the specification

teaches one of skill in the art methods of generating SATACs from any species, including plants and also methods of transforming such SATACs into plant cells for the generation of a transgenic plant. In support of the teachings of the specification, Applicant has provided four Declarations of record, which evidence, based on the teachings of the specification that 1) mammalian SATACs can be introduced into diverse plants cells, **including monocot and dicot species** (i.e., tobacco cells, Arabidopsis cells and rice protoplasts) by microcell-mediated fusion and cationic lipid-mediated transfection (**Declaration 1**); that plant SATACs are generated using the methods as described in the application (**Declaration 2** and further detailed and exemplified by Figures in **Declaration 4**); and that a plant SATAC can be introduced into a plant cells by cell fusion, and grown under conditions that regenerate a transgenic plant containing a satellite artificial chromosome (**Declaration 3**). In addition, as noted, Applicant provides two additional Declarations, Declaration 5 and Declaration 6, to further evidence the universality of the fundamental chromosomal processes as discovered and described in the application, and its applicability to the generation of SATACs in diverse species, including mouse, humans and diverse species of plants. Applicant urges the Examiner to consider these Declarations as a whole, which evidence the operability of the subject matter as claimed based on the teachings of the specification.

Relevant Law

The case law has been discussed in previous responses: To summarize:

Enablement is a legal determination that assesses whether a specification teaches one of skill in the art to make and use what is claimed. As noted enablement is not precluded even if some experimentation is necessary, as long as the amount of experimentation is not undue. Atlas Powder Co. v. E. I. Du Pont De Nemours Co., 750 224 USPQ 409, 3 (Fed. Cir. 1984); W. L. Gore and Associates v. Inc., 721 220 USPQ 303, 315 (Fed. Cir. 1983).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. Smith v. Snow, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

Nothing more than objective enablement is required, and therefore it is irrelevant whether this teaching is provided through broad terminology or illustrative examples. In re Marzocchi, 439 220, 223, 169 USPQ 367, 369 (CCPA 1971). An analysis of whether the rejected claims are supported by an enabling disclosure requires a determination of whether that disclosure contained sufficient information regarding the subject matter of the claims as to teach one of skill in the art how to make and use what is claimed. "[I]t is not a function of the claims to specifically exclude either possible inoperative substances or ineffective reacting proportions." *In Application of Dinh-Nguyen*, 492 F.2d 865 at 858-9 181 USPQ 46 (CCPA (1974)). Thus, a claim is not too broad because it does not explicitly exclude every conceivable unworkable application of the method, providing it enables one of skill in the art to practice what is claimed in its workable applications.

The scope of enablement is based on that which is disclosed in the specification plus the scope of what would be known to one of skill in the art without undue experimentation. *National Recovery Technologies, Inc. v. Magnetic Separation Systems, Inc.*, 166 F. 3d 1190, 49 USPQ 2d 1671 (Fed. Cir. 1999). A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1384, 231 USPQ 81, 94 (Fed. Cir. 1986), *cert. denied*, 480 U.S. 947 (1987); and *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984). Hence all that is known to those of skill in the art is part of the disclosure of the application.

To establish a *prima facie* case of lack of enablement, the Examiner has the initial burden to establish a reasonable basis to question the enablement provided for what is claimed. In re Wright, 999 1557, 1561-62, 27 1510, 1513 (Fed. Cir. 1993). (examiner must provide a reasonable explanation as to why the scope of protection provided by a claim is not adequately enabled by the disclosure). See also Morehouse, 545 162, 192 USPQ 29 (CCPA 1976). The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the subject matter *as claimed.* A considerable amount of experimentation is permissible, particularly if it

is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988). All factors must be considered. A deficiency in meeting some factors does not preclude a finding of enablement. Consideration of a few factors is **not** dispositive.

Analysis

Although the Wands factors listed above have been addressed in detail in previous responses (each incorporated by reference herein), they are reiterated herein to evidence that, when all factors are considered as a whole, no undue experimentation is required to practice the subject matter as claimed. In addition, the Examiner's arguments are rebutted in turn below.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. As discussed in detail below, Applicant respectfully submits that the instant application teaches a method for the generation of a transgenic plant following introduction of a SATAC, including a plant SATAC, into a cell, including plant cells or protoplasts, and the generation of a transgenic plant therefrom. Based on this and consideration of the other Wands factors, it would not require undue experimentation to produce a SATAC, including a plant SATAC, a plant cell with a SATAC or to grow the plant cells under conditions to produce transgenic plants that are within the scope of the claims, in view of the knowledge and level of skill in the art and the teachings and disclosure in the specification regarding methods for generating satellite artificial chromosomes for use in different species. Further, as discussed above with respect to the written description requirement, the SATACs, including plant SATACs, are described and characterized in the specification in great detail. Therefore, one of skill in the art can readily identify, make and use SATACs, plant SATACs and cells containing SATACs without undue experimentation.

As demonstrated below, consideration of the so-called "Wands factors" renders it evident that it would not require undue experimentation to perform the steps of the methods claimed herein, including the preparation and isolation of satellite artificial chromosomes from any eukaryotic species, including plants, their introduction into plant cells such as a

plant protoplast of any species, including monocot and dicot species, or the growth of such plant cells under conditions to produce a transgenic plant containing a satellite artificial chromosome.

1. **The scope of the claims**

The claims are directed to methods for producing a transgenic plant by introducing a satellite artificial chromosome into a plant cell and growing the plant cell under conditions to produce a transgenic plant. The dependent claims specify particular types of plant cells or protoplasts, that the satellite artificial chromosome is a plant satellite artificial chromosome, and/or comprises heterologous DNA that encodes a gene product, and/or particular methods for the introduction of satellite artificial chromosomes into plant cells for the generation of a transgenic plant.

2. Level of skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application address a highly skilled audience and further evidence the high level of skill in this art. Therefore, Applicant respectfully submits that using the teachings of the specification, one of skill in the art, could apply such teachings to making non-animal SATACs, such as plant SATACs, introducing SATACs into numerous cell types, including plant cells and protoplast, and generating transgenic plants therefrom.

3. Teachings of the specification

Applicant respectfully submits that SATACs and plant SATACs are taught by the instant application. As discussed above with respect to the written description requirement, the specification provides descriptions of SATACs and plant SATACs and structural elements thereof. The specification further teaches the exact steps involved in generating SATACs and describes the results of each step so that one of skill in the art need only follow the teachings to obtain SATACs of any species. As taught in the specification and demonstrated in the Declarations of records and the attached Declarations provided herewith, it only is necessary to introduce a piece of DNA into a cell, grow the cell, and look for cells that contain satellite artificial chromosomes. Use of a selectable marker on the piece of DNA renders it easier to find the cells in which the events that lead to the generation of satellite artificial chromosomes occur. Such cells can them be selected, and a satellite artificial chromosome isolated therefrom. The specification teaches methods for isolation of satellite artificial chromosomes. The specification also teaches the introduction of SATACs of any

species into any cells, including plant cells such as protoplasts, for the the generation of transgenic plants therefrom.

1) Production of SATACs including Plant SATACs

The instant application teaches SATACs and methods of making SATACs. As stated in the instant application, the methods provided can be applied to different species (page 12, lines 6-9). Additionally, Applicant is not required to teach what is well known in the art. In re Buchner, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). Thus, it is sufficient if one of skill in the art can make and use the claimed subject matter using the teachings of the specification coupled with information known in the art MPEP §2164.01.

A SATAC, like most chromosomes, is a large structure. In contrast to an individual gene where the DNA sequence is the essence of the gene, it is structure that is the essence of a SATAC. The application teaches that a SATAC is a chromosome that contains more heterochromatin than euchromatin and can include portions of heterologous DNA (see *e.g.*, page 18, lines 24-26; page 19, lines 3-5; page 19, lines 3-8). The instant application teaches the structural elements of a SATAC (see, for example, pages 44-47 and Figures 2-3, which schematically depict that process and chromosomal structures). The specification teaches in detail how to make such SATACs. The specification further teaches identifying elements of a SATAC, *e.g.*, more heterochromatin than euchromatin and other chromosomal structure, such as centromere, telomeres, origin of replication, which result upon application of the methods.

The teachings of the specification are not limited to any particular species of SATACs, but provide SATACS, including SATACs from any species, plant or animal. The specification teaches that a plant SATAC is a SATAC with a plant centromere (page 16, lines 27-29). The inclusion of a plant centromere in the SATAC occurs by virtue of the amplification events, that can include duplication of the centromere, leading to production of a formerly dicentric chromosome. The amplification events occur upon practice of the methods.

Also, the teachings of the specification regarding SATAC structure and elements of SATACs are applicable across species. Further, the structure of chromosomes and elements of chromosomes, such as those found in SATACs, are conserved between and among species and across kingdoms; centromere, telomeres, origins of replication and heterochromatin are recognizable structures across species. The specification also teaches methods known in the art for identifying chromosome structure and elements thereof, such as chromosome banding

(page 72-73), immunolabeling, immunofluorescence (page 73) and scanning electron microscopy (page 73), that are applicable to SATACs of any species.

The specification discloses methods of generating satellite artificial chromosomes (SATACs), and characterizes in exquisite detail the artificial chromosomes generated by such methods. To illustrate the methods and products thereof, the specification describes the exact procedures used to generate multiple specific cell lines containing SATACs (see, *e.g.*, Examples 2-7, beginning at page 75, line 8), and Applicant provides to the public no less than six of the described cell lines that have been deposited at an authorized depository (*i.e.*, the European Collection of Animal Cell Culture) (see, *e.g.*, page 74, line 22, through page 75, line 7).

The specification teaches identification and characterization of the satellite artificial chromosomes and each of the intermediates generated in the process of *de novo* satellite artificial chromosome formation. Extensive analyses using methods including Southern hybridization, long-range mapping of restriction endonuclease sites, indirect immunofluorescence with anti-centromere antibodies provide definition of the artificial chromosome at the level of the basic structural and functional elements that comprise these chromosomes, including the characteristic repeated units of satellite and foreign DNA. Many of these features are depicted schematically in Figures 1-3 of the application.

For example, the specification exemplifies generation of SATACs. As exemplified, the amplification event leads to formation of a sausage chromosome from a formerly dicentric chromosome (see for example, Example 4, pages 83-91, and Figure 2). The specification exemplifies formation of a satellite artificial chromosome (a megachromosome), which can be via an intermeidatesausage chromosome, and more importantly, teaches the structural features of the megachromosome observed with immunofluorescence and with electron microscopy (see for example, Example 6, pages 92-97). Events leading to formation of a SATAC occur by following the teachings of the specification (*i.e.* introducing heterologous nucleic acid into a cell, growing the cell and identifying cells containing multicentric, such as dicentric chromosomes, and generating satellite artificial chromosomes from the formerly dicentric chromosomes.

By following these teachings, one of skill in the art can produce and recognize a SATAC from any species, including a plant and produce and recognize a SATAC. Furthermore, at the time of the effective filing date of this application, there was a lot of knowledge in the art for introduction of DNA into plants, such that by following the

teachings of the specification those of skill in the art could readily produce SATACs in any species of animal or plant cell based upon the teachings in the specification.

The underlying events upon which the generation of satellite artificial chromosomes relies are not unique to an particular species of eukaryotic cell. On the contrary, the specification teaches that the events and the methods based thereon are applicable to cells from any eukaryotic species. As noted above, the generation of a satellite artificial chromosome requires an amplification event. Such an event occurs in all eukaryotic chromosomes, animals and plants. In fact, plants have long been known to have amplifiable regions (see *e.g.*, U.S. Patent Nos. 6,355,860 and 6,100,092, which demonstrate this assertion; see Borysuk *et al.* (1988) *Theor Appl. Genet* 76:108-112 and Borysuk *et al.* (1993) *Plant Mol. Biol.* 21:381-384). Further, as discussed above with respect to the Written Description rejection and further below, the Declaration 6 of Hadlaczky shows that the process of generating SATACs is universal among species, and can occur in plants and mammals, including humans.

The applicability of the teachings of the specification for making and identifying plant SATACs is further evidenced by the Declaration 2 and 4 of Fabijanski of record and Declaration 5 of Fabijanski, provided herein. The Declarations demonstrates that by following the teachings of the specification, one of skill in the art can generate a plant SATAC, which is identified by its features as taught in the specification. For example, as shown by the Declaration 5, one can introduce heterologous DNA with homology to pericentric DNA to generate a chromosome with a "sausage amplification" and a plant SATAC. The Declaration 5 shows generation of these exemplary structures in two distinct plant species. The plant SATACs generated are recognized by structural features of SATACs as described in the specification, by the identification of precursor structures, such as dicentric chromosomes, sausage chromosomes and then SATACS, just as depicted and described in the instant application and all priority applications. The existence of the precursor and intermediate products shows that the amplification events leading to generation of SATACs occurs in plants as taught in the specification and results in SATACs as taught in the specification. This Declaration indicates that the Declarant and those working with have produced numerous different SATACs in at least two plant species.

Therefore, the specification teaches one of skill in the art how to generate satellite artificial chromosomes from various species (*e.g.*, plant and animals); readily identify the resulting satellite artificial chromosomes based on the detailed characterization provided in

the specification; incorporate foreign nucleic acid (*e.g*, heterologous DNA encoding a product into an artificial chromosome); isolate and transfer artificial chromosomes into cells from various species (*e.g*., plants and animals). Thus, the teachings of the specification provide how to make and use the satellite artificial chromosomes and to combine these artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the introduction and long-term expression of nucleic acids encoding products in cells of host animals, plants and insects.

2) Introduction of SATACs into cells

The specification teaches numerous methods for introducing SATACs into a cell, and the use of such SATACs for the generation of transgenic plants and the production of protein into cells (*e.g*., page 11, lines 1-7.) Such methods include direct DNA transfer, electroporation, lipid-mediated transfer, e.g., lipofection and liposomes, microprojectile bombardment, and microinjection (see for example, pages 48-56). The specification teaches that chromosomes can be transferred by preparing microcells containing a satellite artificial chromosome and then fusing with selected target cells. Cell fusion can be used to transfer SATACs. Cell fusion also is exemplified in Example 1. Further, based on the teachings of the specification, the Declarations of record evidence the introduction of SATACs into plant cells and the generation of a transgenic plant. For example, Declaration 1 evidences introduction of a mammalian SATAC into tobacco and Arabidopsis protoplasts by microcell-mediated fusion and into rice protoplasts by cationic lipid-mediated transfection. Also, Declaration 3 evidences the introduction of a plant SATAC into plant cells by cell fusion resulting in the generation of a hybrid transgenic plant.

The specification also teaches methods for introducing SATACs into cells by producing satellite artificial chromosomes in cells (see *e.g*, page 6, lines 17 to page 7, line 26). For example, the specification teaches methods for producing satellite artificial chromosomes that contain heterologous DNA and the expression of the heterologous DNA contained therein in cells, for example, for the expression of gene products (see, e.g., page 39, line 25, through page 41, line 3; page 61, line 28, through page 62, line 7; page 150, line 1 through page 165, line 12 and Example 12 beginning on page 140).

The specification further teaches that the SATACs introduced can be isolated or purified, or can be in the form of microcells or other such form for fusion with recipient cell (see *e.g*, page 41, line 4 through page 42, line 3; page 32, lines 13-24; page 80, lines 20-27;

and Example 10 beginning on page 124). For example, the specification at page 41, line 4 to page 42, line 3 describes a variety of methods that can be used to isolate SATACs such as, for example, fluorescence activated cell sorting (FACS), swinging bucket centrifugation, zonal rotor centrifugation, velocity sedimentation, and affinity-based methods. Preparation of microcell containing SATACs also is described and taught in the specification (see e.g, page 51, lines 15-26).

3) Generation of transgenic plants

Methods of producing a transgenic plant from a plant cell such as a plant protoplast were well known to those of skill in the art at the time of filing of the above-captioned application and as of its earliest priority date. As the specification recites, the method used for producing a transgenic plant is primarily a function of the species of plant (exemplary species of plant are provided in the specification, see, e.g., page 54, lines 12-16) and protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art. Further, the specification incorporates by references, methods of producing transgenic plants (see e.g., U.S. Patent Nos. 5,489,520 and 5.482,928). Further, as described above, Declaration 3 evidences the generation of a hybrid transgenic plant by introducing a plant SATAC into a plant cell by cell fusion based on the teachings of the specification.

Thus, the teachings of the specification provide how to make and use the satellite artificial chromosomes and to combine these artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the introduction and long-term expression of satellite artificial chromosomes in eukaryotic cells, including plant cells, and the generation of transgenic plants from these cells.

4. Knowledge of those of skill in the art

As of the effective filing date of the application, a broad body of knowledge was available about properties and structural components of chromosomes. Such knowledge included availability of structural and sequence information regarding plant heterochromatic pericentric DNA. As noted, however, the methods taught in the application do not require knowledge of any plant chromosomal sequences.

As described in the specification, such as by citation of numerous references, those of skill in the art knew of methods of DNA transfer into cells and between different cell types. Also known to those of skill in the art were methods for the manipulation of DNA,

recombinant DNA techniques, techniques for the transfer of DNA into cells, including plant cells, and the production of transgenic plants from plant cells such as protoplasts containing heterologous DNA. Many examples of such knowledge are cited in the instant application and in the instant response.

Elements of SATACs, identified in the instant application, were available in the art for SATACs, including plant SATACs. For example, if targeted introduction of DNA into the pericentric region of a plant chromosome was desired, pericentric DNA sequences in plant DNA were known, see for example, Genbank Accession No. X52320; submitted 25 April 1990; Schmidt et al. Science (1995) 270:480-83; Murata *et al.* (1994) Jpn. J. Genet. 69:361-70. Also, as set forth in the Declaration 5 of Fabijanski provided herewith, it was also known in the art that rDNA sequences are found within pericentric heterochromatin (see *e.g.*, Hizume M *et al.* (1992) *Jpn. J. Genet.* 67: 389-396; Sato *et al.* (1980) *Cytologia* 45:87-96; and Maluazynska J and Heslop-Harrison JS (1991) *Plant J.* 1:159-166). As described in the application, including the priority application, targeted introduction is not needed, and, in fact, was not employed in generating exemplary SATACs in the specification. Also, not needed, but known to those of skill in the art by the time of the priority date of the instant application were sequence information for plant centromeres, telomeres and autonomously replicating sequences (ARS) (see e.g., Richards, E.J. "Plant Telomeres" in *Telomeres* Eds. C. Greider, and E.H. Blackburn, Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1995); Rhodes *et al. Cur. Opin. Struct. Biol.* 5(3): 311-322 (1995); Zakian Science 270(5242):1601-1607 (1995); Berlanki et al. Plant Mol. Biol. 11:161-162 (1988); Berlani et al. Plant Mol. Biol. 11:173-182 (1988); and Eckdahl et al. Plant Mol. Biol. 12:507-516 (1989)).

Techniques and materials for plant transformation for use with SATACs were also available in the art. Numerous techniques for introducing DNA into plant cells were known, for example, Uchimiya *et al.* (1989) *J. of Biotech.* 12:1-20; Weissbach *et al.* (1988) *Methods for Plant Molecular Biology*, Academic Press, N.Y., Section VIII, pp. 421-463; Grierson *et al.* (1988) *Plant Molecular Biology*, 2d Ed., Blackie, London, Ch. 7-9, Miranda *et al.* (1992) *J. Bacteriol.* 174:2288-97 and for example, U.S. Patent Nos. 5,436,392; 5,489,520; 5,470,708; 5,491,075; 5,482,928; and 5,424,409. Numerous techniques were also available for use in transferring SATACs from one cell into another, for example, Parkonny *et al.*, (1992) *Plant J.* 2:863-74; Constabel F. (1976) *In Vitro* 12: 743-8'; Jones *et al.* (1976) *Science* 194:401-03; Cocking (1984) *Ciba Found Symp.* 103:199-28. Plant selectable markers were

available, such as phosphinotrhicin acetyl transferase and hygromycin (see for example, White *et al.* (1990) *Nuc. Acids Res.* 18:1062; Spencer *et al.* (1990) *Theor. Appl. Genet.* 79:625-631; Vickers *et al.* (1996) *Plant Mol. Biol. Reporter* 14:363-368; Thompson *et al.* (1987) *EMBO J.* 6:2519-2523; and Blochinger and Diggelmann, *Mol. Cell. Biol.* 4:2929-2931.

Techniques for identifying SATACs as described in the instant application were available for use with SATACs, including plant SATACs, and plant cells. For example, chromosome banding and labeling, Wang & Fedoroff (1972) *Nature* 235:52-54, Sumner (1972) *Exp. Cell Res.* 75:304-306, Perry & Wolff (1974) *Nature* 251:156-158; Immunolableing, Hadlaczky *et al.* (1986) *Exp. Cell Res.* 167:1-15, Hadlaczky *et al.* (1991) *Proc. Natl. Acad. Sci.* U.S.A. 88:8106-8110; electron microscopy, Sumner (1991) *Chromosoma* 100:410-418. Such techniques were known to one of skill in the art and had also been demonstrated as applicable with plant chromosomes as well as with plant cells (see for example, Sparvoli *et al.* (1994) *J. Cell Science* 107: 3097-3103 and Wanner *et al.* (1995) *Chromosome Res.* 3:368-74).

Methods for introducing heterologous DNA into plant cells and generating transgenic plants from the transformed cells were known in the art at the time of filing and before. For example, as of the earliest priority date of the above-captioned application, it was recognized that a variety of plant cells, including plant protoplast, were versatile tools for introducing heterologous DNA, monitoring gene expression in the transformed cells, conducting developmental studies and generating transgenic plants. As of the earliest priority date of the above-captioned application, those of skill knew that plant cells could readily be transformed and induced to generate a variety of plants (see, *e.g.*, Negrutiu *et al.*, *Int. J. Dev. Biol.* 36:73-84 (1992); Rogers *et al.*, "Methods for Plant Molecular Biology," Academic Press (1988) VIII (26):423-436; Zupan *et al.*, *Proc. Natl. Acad. Sci.* USA, 93:2392-2397 (1992)). As the specification states, the method used for producing a transgenic plant is primarily a function of the species of plant. At the time of filing, numerous protocols were available for producing transgenic plants. The protocols used to introduce DNA into plant cells and produce a transgenic plant are readily adapted by one of skill in the art.

These references to numerous published information and protocols regarding plant and animal chromosomal composition and structure, DNA manipulation, recombinant DNA expression, transfer of DNA into cells, including plant cells, and production of transgenic plants demonstrate the large volume of information regarding tested and reliable procedures

available at the time of filing of the instant application. This evidences the advanced state of the art at the relevant time and the availability of such procedures for manipulation of plant cells and generation of satellite artificial chromosomes into such cells, and introduction of satellite artificial chromosomes into plant cells to to produce a transgenic plant from the satellite artificial chromosome-containing plant cells.

5. Presence of Working Examples

The specification provides numerous working examples and descriptions of the generation, isolation and transfer of SATACs from various sources. Such examples are exemplary, not only for animal SATACs and SATAC introduction into cells, but also for the construction of SATACs from other species, including plants.

The working examples of animal SATACs provide sufficient teachings, in combination with what was known to those of skill in the art at the time of the instant application's earliest priority date, to generate, identify and transfer a SATAC, regardless of the organism from which it is derived or the cell type to which it is introduced. For example, techniques for use in the generation and identification of SATACs are provided in Example 1, such as chromosome banding and labeling methods, cell fusion and microcell fusion methods, and DNA methods (see for example, pages 69-74). The specification also provides detailed examples of SATAC formation including descriptions of intermediary structures such as dicentric chromosomes and sausage chromosomes (see, for example, Example 4 which describes formation of a sausage chromosome from a formerly dicentric chromosome). The specification provides examples of SATAC production from such intermediates (see, for example, Example 6, which describes production of a megachromosome from the sausage chromosome at pages 92-93), and additionally provides schematic representations of SATAC formation and exemplary SATACs in Figures 2 and 3. Structural features of exemplary SATACs, such as megachromosomes, are also described (see, for example, pages 93-97).

Example 2, at page 75 of the specification, describes in great detail the preparation and maintenance of cell lines, including EC3/75, EC3/7C5 and EC3/7C6, which contain artificial chromosomes, as well as the assays used to monitor the expression of the *neo* gene encoded by the artificial chromosomes within the cells. Example 6, at page 92 of the specification, describes in great detail, methods for the generation of cell lines containing a megachromosome and detailed structural characteristics of this satellite artificial chromosome. Example 8, at page 113 of the specification, describes in great detail the *in vivo* replication of a megachromosome. Example 10, at page 124 of the specification,

describes in great detail methods for the isolation of satellite artificial chromosomes from endogenous chromosomes based upon the atypical base content and/or size of the satellite artificial chromosomes. Example 12, at page 140 of the specification, describes in great detail the preparation of vectors and plasmids, such as the λCF-7 and the λCF-7-DTA vectors and the pMCT-RUC and the pLNCX-ILRUC plasmids, for the targeted integration of heterologous DNA into artificial chromosomes. Example 13, at page 165 of the specification, describes methods for the microinjection of artificial chromosomes into eukaryotic cells, and detection of expression of the encoded heterologous DNA (βgal) in cells injected with the DNA.

Although the working examples exemplify the teaching in mammalian cells, the teachings are directly applicable to other species, including plant cells when placed in the context of the instant application in its entirety, which provides further guidance for other SATACs, including human and plant SATACs. Further, Applicant is not required to provide data or illustrative examples in support of every assertion in the specification of everything within the scope of a broad claim. In re Anderson, 176 USPQ 331, at 333 (CCPA 1973)). Nonetheless, as discussed below, the accompanying Declaration 6 demonstrates that by following the teachings of the instant application, which are exemplified in the working examples, one of skill in the art can universally generate SATACs, and maintain SATACs. Other Declarations of record, and Declarations 5 and 6 provided herewith, demonstrate introduction of SATACs into diverse cell types, including plant cells and human cells. Declaration 6 further evidences the universality of the underlying chromosomal events and of the methods and disclosure of the application.

6. Predictability

Using the teachings of the specification and given the level of skill and the knowledge of those of skill in the art, it would not be unpredictable to make a SATAC, such as a SATAC from any eukaryotic species, including a plant SATAC, nor to transfer a SATAC into a plant cells to generate a transgenic plant therefrom. As discussed above in great detail, the specification teaches methods for making SATACs, methods for identifying SATACs, including the elements of SATACs, and exemplary structures, and methods of introducing SATACs into cells and generating transgenic plants therefrom. As the specification provides and as explained above, these teachings are applicable to non-animal SATACs, including plant SATACs. Declaration 5 shows that the methods are reproducible, since a number of different SATACs were generated in at least two different plant species. In addition,

Chromos Molecular, an assignee of the instant application, markets SATACs and their uses for production of transgenics, gene therapy and gene products. The basic methods employed are fundamentally the same as in the original application.

The Office Action appears to imply that the function of SATACs are unpredictable because of the alleged rapid degradation of foreign DNA molecules that occurs in cells upon transformation as set forth in Ohgawara et al. The Examiner further appears to urge that in view of this unpredictability it would have required undue experimentation to develop a multitude of SATACs from a multitude of non-exemplified animal and plant species and to evaluate the persistence of these SATACs in plant cells following lipid-mediated transfection or any other means. The Office provides no evidence to support this, and the Declarations of record demonstrate that this concern is not valid.

As indicated in responses of record, the application describes that **SATACs are fully functional stable chromosomes that are a stable extra-genomic chromosomal system** (see e.g., page 5, lines 14-19; page 6, lines 5-6; page 62, lines 5-7). The Examiner has provided no sound evidence to doubt this assertion. It is respectfully submitted that the Examiner's reliance of Ohgawara *et al.*, which describes the instability of transformed DNA and not an artificial chromosome structure, is misplaced. In fact, as noted in the response filed November 09, 2005, the limitations of DNA maintenance as set forth in Ohgawara *et al.* is overcome by the stable autonomous replication of satellite artificial chromosomes.

In addition, the Examiner's comments regarding Applicant's *allegations* of the lack of a requirement for genomic integration of autonomously replicating SATACs with respect to enablement of the broadly claimed genus of SATACs (see bottom of page 14 to top of page 15 of the instant Office Action mailed March 30, 2006), appears to imply that the Examiner doubts Applicant's assertion that **all** SATACs are stable and autonomously replicating and do not integrate into the host genome. First, Applicant respectfully submits that this is not an allegation but is a functional characteristic of a SATAC. The specification describes that SATACs, including SATACs from any species, are a stable extra-genomic chromosomal system. This is described generically in the application and exemplified with respect to a mouse SATAC, and demonstated in Declarations of record.

Declarations of record, based on the teachings of the specification, further evidence the stability and the presence of a distinct chromosomal structure that do not integrate into the host genome. For example, Declaration 1 of Fabijanski describes the transfer of a mammalian SATAC into tobacco and Arabidopsis by microcell-mediated fusion and into rice

protoplasts by cationic lipid-mediated transfections and detection of the artificial chromosomes 8-16 weeks later. This evidences the stability of the mammalian SATAC in the cell. Further, Declaration 3 of record evidences the generation of a hybrid transgenic plant by introduction of a plant SATAC by cell fusion, and the regeneration of 50 hybrid plants expressing the marker genes expressed from the SATAC. The regeneration of the plants and the expression of the marker gene evidences the stability of the SATAC in the plant cells. In addition, the presence of the SATAC structure as a distinct chromosome with no requirement for genomic integration is depicted in Declaration 4 of Fabijanski and Declaration 5 provided herewith. For example, Figure 3 of Declaration 4 depicts a small, independent chromosomal structure (indicated with a yellow arrow in the DAPI stained image set forth in Figure 1). Also, Figure 3 of Declaration 5 provided herewith depicts a SATAC, identified based on the substantial amount of pericentric heterochromatic DNA and the presence of the selectable marker, on a distinct chromosome structure. Thus, one of the apparent challenges in plant transformation at the effective filing date was stable maintenance of foreign DNA in transformed plants which, as described in the application, and further demonstrated in the Fabijanski Declarations, is overcome by use of SATACs. Therefore, unpredictability is not a factor weighing against enablement.

Conclusion and summary

Therefore, based upon consideration of all of the factors, it would not require undue experimentation to prepare SATACs from any species, including plants, and to use such SATACs to introduce into plants cells for the generation of a transgenic plant. The generation of SATACs is thoroughly described and taught in the subject specification. The specification describes in extensive detail the preparation, characterization and isolation of satellite artificial chromosomes and provides numerous examples of particular embodiments thereof. Declarations of record and provided herewith demonstrate the practice of the methods described in the application results in preparation of SATACs from any species, including mouse, human and diverse plant species, and evidence the generation a transgenic plant by introducing a SATAC into a plant cell as claimed. Hence, the Declarations also evidence the predictability of the methods. The level of skill and knowledge of those of skill in the art is high, and the prior art describes all methods needed to practice the methods as claimed in accord with the teachings of the specification.

In summary, one of skill in the art, by following the methods set forth in the specification can generate satellite artificial chromosomes and readily identify the resulting

satellite artificial chromosomes based on the detailed characterization provided in the specification. Further, by following the methods set forth in the specification, one of skill in the art can isolate and introduce satellite artificial chromosomes into plant cells. By virtue of Applicant's discovery of satellite artificial chromosomes and the teachings of the specification, those of skill in the art are able, to make and use satellite artificial chromosomes and to combine the satellite artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the generation of transgenic plants containing the satellite artificial chromosomes. Clearly, Applicant's discovery of a means of generating artificial chromosomes, the basic functional units common to all eukaryotes for the storage and transmission of vital genetic information, which are maintained extra-genomically in host cells but function in the same manner as endogenous chromosomes, has broad and immediate applicability in the field of recombinant DNA. Applicant is entitled to claims of a scope commensurate with the far-reaching development which has been provided to the public for immediate and valuable use through the guidance of the instant specification.

Fairness

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. In the above-captioned application, Applicant discloses to the public pioneering methods and compositions for the controlled introduction and stable extra-genomic maintenance of large heterologous DNA fragments in cells, and the generation of transgenic plants therefrom. It is clear that Applicant's discovery is of a pioneering nature, and, as such, is entitled to broad claim protection. It would be unfair and contrary to the public policy and to the Constitutional mandate set forth in Article, Section 8, to require Applicant to limit the claims to methods using mouse SATACs. To do so would permit those of skill in the art to practice what is disclosed in the application but avoid infringing so-limited claims. To so limit the claims is contrary to the public policy upon which the U.S. patent laws are based. See, for example, *In re Goffe*, 542 F.2d 801, 166 USPQ 85 (CCPA 1970):

> for the Board to limit appellant to claims involving the specific materials disclosed in the examples so that a competitor seeking to avoid infringing the claims can merely follow the disclosure and make routine substitutions "is contrary to the purpose for which the patent system exists - to promote progress in the useful arts."

The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the disclosure. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions. *In re Sus and Schafer*, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301, at 304.

As noted above, the technology disclosed in the instant application and priority applications is of a pioneering nature and a cornerstone of Chromos Molecular Systems, Inc. which was founded to exploit this technology, and which has numerous commercial ventures based on this technology. To limit the claims or not grant claims, could permit Chromos' partners and collaborators, and licensees, to exploit the technology developed by Chromos without the need for licenses. To require Applicant to so-limit the claims would deprive Applicant of protection of the broad applications of the pioneering discovery disclosed and described in exhaustive detail in the subject application. Applicant therefore respectfully submits that the claims directed to SATACs, including plant SATACs, and the use of such SATACs to generate a transgenic plant are commensurate in scope with the discovery and its disclosure within the above-captioned application.

DECLARATIONS

Notwithstanding the above arguments, Declarations 5 and 6 are provided herewith to provide evidence of the pioneering nature and broad scope of the disclosure of the application and priority applications.

1) DECLARATION 5

To evidence that generation of plant SATACs using methods as taught in the instant application, attached is a Declaration 5 under 37 C.F.R. §1.132 of Steven F. Fabijanski. The Declaration details construction of plant SATACs in two distinct plant species, *Nicotiana* and *Brassica*. Declaration 5 shows that by following the teachings of the application as of its earliest filing date, plant SATACs can be generated and maintained in plant cells. It also it noted that Declaration 5 does not introduce the 334 bp tobacco sequence that was introduced as heterologous DNA in Declaration 4 (and Declarations 2 and 3). In neither instance, is such sequence used to generate a plant SATAC. The Arabidopsis rDNA sequence and the selectable marker used were the same in the studies in both Declarations.. Hence, the Declaration 5 shows that the 334 bp sequence is not required to generate a plant SATAC.

Dr. Fabijanski is not an inventor of this application, he is a Ph.D. Since those of skill in the art typically have advanced degrees, Dr. Fabijanski, who has a Ph.D. degree, is

representative of a person of skill in this art with respect to performing experiments in accord with a disclosed protocol. It is noted that he is an employee of Agrisoma, a company in which Chromos has an ownership interest, and which is a licensee of the instant application. In performing or directing the experiments in Declaration 5, he, and others under his direction and control, followed the teachings in the application.

Specifically, the Declaration 5 of Dr. Fabijanski demonstrates that by following the teachings in the application, plant SATACs can be generated by i) introducing a DNA fragment with a selectable marker into a plant cell (in this case either *Nicotiana* protoplasts or *Brassica napus* protoplasts); ii) growing the cell under selective conditions to produce plant cells that have incorporated the DNA into their genomic DNA such that a plant SATAC is produced; and iii) selecting a cell that contains a plant SATAC. The plant SATAC contains a plant centromere, as well as amplified pericentric DNA and the introduced heterologous DNA.

Heterologous DNA containing homology to the pericentric region of plant chromosomes, a selectable marker, either phosphinothricin N-acetyltransferase (PAT) gene or phosphinothricin acetyl transferase gene (*bar)*, were introduced into *Nicotiana* protoplasts or *Brassica napus* protoplasts, respectively. Following selection of cells, fluorescence in situ hybridization (FISH) was used to demonstrate the formation of a sausage chromosome, and a resulting plant SATAC in both species of plants. As set forth in the Declaration 5, no knowledge of the plant centromere sequence, nor any plant sequence, is required. As taught in the above-captioned application, the plant SATACs were generated following amplification and the generation of a *de novo* centromere that occurs upon integration of a DNA fragment into the pericentric heterochromatin. The results of these analyses demonstrate that the methods described in the above-referenced application can be used to generate, identify and maintain plant SATACs in plant cells as taught in the application, including the ultimate parent application.

2) DECLARATION 6

As discussed above, a Declaration 6 under 37 C.F.R. § 1.132 of Dr. Gyula Hadlaczky, an inventor of the claimed subject matter, is provided herewith. The Declaration 6 is provided to evidence the universality of the underlying chromosomal processes involved in the generation of SATACs and of the process for production of SATACs described in the application and the SATACs described in the application. The Declaration demonstrates that by following the teachings of the application, SATACs from diverse species, plants and

mammals, have been prepared by the methods taught in the specification. The Declaration 6demonstrates that the methodology generically described in the application and exemplified with rodent chromosomes, is reproducible. Further, the Declaration 6 points out that the methods disclosed in the application are based an universal amplification events common to all eukaryotic chromosomes. Based on data provided in the Declaration 6 and the accompanying Declaration 5 of Fabijanski, the universality is evidenced by the fact that SATACs can be prepared in accord with the teachings of the application in species as diverse as mammalian species and plant species. Surely, if the amplification occurs in plants, humans and rodents, it is can be inferred that it is a universal phenomena that occurs in other mammals, including whales and gorillas and dolphins and rats and apes.

The Declaration 6 describes the results of the use of methods taught in the above-captioned application for the generation of satellite artificial chromosomes from human chromosomes. These results demonstrate that the methods of artificial chromosome production taught in the above-captioned application are broadly applicable and can be used to generate satellite artificial chromosomes from varied species of organisms.

Specifically, the Declaration 6 of Hadlaczky describes the generation of satellite artificial chromosomes from human chromosomes through the introduction of foreign DNA including a selectable marker into human/hamster hybrid cells containing human chromosomes. This Declaration 6 also incorporates Declaration 5 of Fabijanski, showing the generation of SATACs in plants.

In the demonstration described in Declaration 6, the hybrid recipient cell line, referred to as 94-3 cells, is a fusion of human lymphoblasts and Chinese hamster ovary cells and contains human and hamster chromosomes. Thus, as described in the above-captioned application, cells for use in generating artificial chromosomes can be any variety of cells and are not limited to mouse cells.

As also described in the Declaration 6, the 94-3 cells were transfected using standard calcium phosphate DNA precipitation methods with foreign DNA that included a selectable marker gene. The selectable marker gene was the puromycin N-acetyl transferase gene, which is among the several exemplary selectable markers referred to in the subject application. The remainder of the foreign DNA that was introduced into the 94-3 cells included the β-galactosidase gene, the expression of which may be easily detected, and mouse rDNA. The transfectants were cultured under selective conditions (*i.e.*, in the presence of puromycin), and cells that contain artificial chromosomes were selected in

accordance with the methods set forth in the subject application. Specifically, using standard analytical techniques (*i.e.*, Southern hybridization, LacZ staining, C-banding and *in situ* hybridization) as taught in the application, the selected cells were analyzed to identify those that contain artificial chromosomes.

Declaration 6 describes the results of such analyses that were used to identify, for example, a human satellite artificial chromosome and a human gigachromosome, which is a precursor thereof, in the selected transfectants. Southern hybridization results revealed that greater than 40% of the transfectants contained a high copy number of the pBabe Puro (described in the instant application in Example 12) construct DNA which indicates an amplification of the integrated construct such as can occur in the development of an artificial heterochromatic chromosome. Greater than 30% of the transfectants also expressed β-galactosidase at levels detectable by LacZ staining.

To detect the amplified heterochromatic regions of any of the transfectant chromosomes that had undergone a large-scale amplification following introduction of the foreign DNA, C-band staining of the cells was conducted to specifically visualize constitutive heterochromatin. The results of C-banding analyses revealed that 30% of the transfectants contained amplified heterochromatic segments. In this manner, it was possible to detect sausage chromosomes with a characteristic extended heterochromatic arm just as described in the above-captioned application (see, *e.g.*, page 72, line 28, through page 73, line 4, of the application). *In situ* hybridization of selected transfectant cells with human genomic DNA and human alpha satellite DNA probes confirmed that the sausage chromosome was formed on a human chromosome. Further *in situ* hybridization studies of these cells using pBabe Puro and rDNA probes indicated that these foreign genes co-amplified in the heterochromatic arm of the sausage chromosome as is described in the above-captioned application in general and in the specific example of the analysis of a mouse sausage chromosome (see, *e.g.*, Example 4 at page 83).

Similarly, Declaration 6 describes the identification of human satellite artificial chromosomes and precursors thereof in the transfected cells. C-band staining of the selected transfectants revealed satellite artificial chromosomes containing two heterochromatic arms resulting from continued amplification of a sausage chromosome. *In situ* hybridization of the selected transfectants with human alpha satellite DNA and pBabe Puro probes confirmed that the artificial chromosome had human origins and contained the integrated foreign DNA. Thus, the results of these analyses are as described in the subject application in general and in

the specific example of the analysis of a mouse megachromosome. *In situ* hybridization of the selected transfectants with a pBabe Puro probe also revealed the presence of a human gigachromosome showing characteristics such as those described in the above-captioned application in general and in the specific example of the mouse gigachromosome.

Therefore, the Declaration 6 demonstrates that the disclosure in the above-captioned application provides methods for production of satellite artificial chromosomes in any species and provides satellite artificial chromosomes from varied species, thereby, demonstrating that the unsupported assertion in the Office Action that the specification is enabling only for mammalian, particularly mouse, satellite artificial chromosomes, is not correct. Further, it demonstrates that the process is universal and reproducible and can be successfully applied to many species, including plants. As stated in the Declaration 6 "the process by which SATACs are generated is a universal process, fundamental to replication and recombination in cells." In fact, Dr. Hadlaczky was awarded the prestigious Széchenyi award in 2000 for the work that is the subject of the above-captioned application.

Declaration 6 demonstrates element-for-element and step-for-step that, by following the teaching in the application, one of skill in the art can, without undue experimentation, introduce any heterologous DNA that into any cell, and generate and identify any resulting cells that can contains a satellite artificial chromosome.

Rebuttal to specific arguments set forth in the Office Action regarding scope of enablement

As discussed above, Applicant respectfully submits that a consideration of the factors enumerated in In re Wands leads to the conclusion that the specification teaches one of skill in the art to make and use subject matter that is commensurate with the scope of the claims, without undue experimentation. Applicant additionally addresses specific points raised in the Office Action.

1) The specification teaches, and the Declarations further evidence, the introduction of SATACs into plant cells commensurate in scope to the claimed subject matter

The Examiner states that the Applicant's specification is silent on monocot transformation or the particularly claimed transformation methods. Responsive to the previous response filed November 09, 2005, the Examiner is not persuaded by the Fabijanski Declaration 1 of record evidencing transformation methods as taught in the specification and claimed, including cationic lipid-mediated transfection and cell fusion. The Examiner maintains that it is unclear whether "cationic lipid-mediated transfection" as utilized in the

Fabijanski Declarations is the same as "lipid-mediated transfection as instantly claimed, or whether the instant specification provides enabling support for either. Further, it appears that the Examiner continues to urge that "direct DNA transfer," as originally stated by Potrykus is the most efficient technique for transforming plants.

It is respectfully submitted that the specification is replete with methods of introducing SATACs into cells, including plant cells that are inclusive of monocot and dicots. Such methods include direct DNA transfer, electroporation, lipid-mediated transfer, e.g., lipofection and liposomes, microprojectile bombardment, and microinjection, cell fusion and microcell fusion (see pages 48-56). A patent need not teach, and preferably omits, what is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). Methods of plant delivery techniques were known to one of skill in the art at the time of filing the instant application. For example, each of Ohgawara et al. and Potrykus cited by the Examiner evidence techniques for plant delivery, including lipid-mediated transfection using liposomes, direct DNA transfer, microprojectile bombardment using a particle gun, microinjection, electroporation and others. Although Potrykus notes that many of these methods have not resulted in transgenic offspring, Potrykus suggests this is due to the inability of many of these techniques to facilitate integrative transformation (see page 542, top left column). As described in the specification, and in detail above, an advantage of SATACs is that they are stable and extra-genomic; there is no requirement for genomic integration. Thus, SATACs offer an advantage over gene delivery methods.

In addition, numerous other references directed to delivery techniques in plants were known to one of skill in the art at the time of filing the instant application. These include, but are not limited to, Vain et al. (1995) Biotechnology Advances, 13: 653-671 (cited by Applicant in the response filed November 19, 2005); Klein et al. (1988) Proc. Natl. Acad. Sci., 85:8502-8505; Lee et al. (1991) Proc. Natl. Acad. Sci., 88:6389-6393; Saudners et al. (1995) Mol. Biotechnol., 3:181-90. Also, it also was well known in the art that cation lipid-mediated transfection is one type of lipid-mediated transfection. The term lipofection is used in the application (see page 11, line 3), which is a term well-known in the art at the time of filing to encompass cationic lipid-mediated transfection (see Felgner et al. (1987) PNAS, 84: 7413-7; Felgner et al. (1993) Methods in Cell Science, 15:63-68). For example, the use of Lipofectin, which is a cation-lipid mediated reagent, was well known at the time of filing the application for delivery of DNA to plants (see Sporlein et al. (1991) TAG Theoretical and

Applied Genetics, 83: 1-5). In addition, any of the above techniques are applicable to both monocot and dicot species as described in several of the papers cited above.

Furthermore, it appears that the Examiner is implying that only direct DNA transfer is enabled because it is the most efficient. In response, Applicant respectfully submits that this is not the proper standard for enablement. To satisfy the requirement of enablement, a claim does not have to explicitly exclude every conceivable inactive variant. ("[I]t is not a function of the claims to specifically exclude either possible inoperative substances or ineffective reacting proportions". In Application of Dinh-Nguyen, 492 F.2d 865 at 858-9 181 USPQ 46 (CCPA (1974)). Rather, the question is whether by following the teachings of the application, one of skill in the art can practice what is claimed with perhaps routine, but not undue, experimentation. Not all embodiments must be operative, so long as one of skill in the art can practice the method as claimed without undue experimentation.

In addition, the Declarations of record evidence the delivery of SATACs into cells using methods as taught in the application. For example, Declaration 1 describes the introduction of a mammalian SATAC into tobacco and Arabidopsis protoplasts by microcell-mediated fusion and into rice protoplasts by cationic lipid-mediated transfection. Rice is a monocot plant species. **Hence, as taught in the application, Applicant has introduced a SATAC into a monocot**. Further, Declaration 3 describes the introduction of a plant SATAC into a plant by cell fusion to generate a hybrid transgenic plant.

The USPTO has released "Guidelines for Examination of Applications for Compliance with the Utility Requirement" [guidelines, which address utility under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph] and an "Overview of Legal Precedent Governing the Utility Requirement" [legal overview] to support the guidelines. Under section I.B.4. of these guidelines Examiners are reminded that they must treat as true credible statements made by an applicant or a declarant in the specification or in a declaration provided under 37 CFR §1.132, unless they can show that one of ordinary skill in the art would have a rational basis to doubt the truth of such statements. Thus, the Examiner must accept as true any credible statement made by the Appellant and may only challenge the statement upon a showing that those of skill in the art would consider the assertion **to have no reasonable scientific basis.**

Finally, the Examiner's reliance on *Genentech, Inc. v. Novo Nordisk, A/S*, 42 USPQ2d 1001, 1005 (Fed. Cir. 1997) is inapt regarding the enabling aspects of monocot transformation or the particularly claimed transformation methods. *Genentech, Inc. v. Novo*

Nordisk is directed to whether the disclosure in the Genentech patent at issue enabled the use of cleavable fusion expression to make hGH without undue experimentation. In this instance, cleavable fusion protein expression systems had not previously been applied to human proteins, and no one had been able to produce any human protein via cleavable fusion expression at the time the application was filed. Thus, the court ruled that the question was whether **there was undue experimentation** to practice the subject matter as claimed when there was no disclosure of any specific starting material.

As described above, and after a consideration of **all** of the Wands factors, including the teaching in the specification, the knowledge of skill in the art and other factors, it is respectfully submitted that the application enables transformation methods generally, and transformation of monocots. Unlike the case in *Genentech, Inc. v. Novo Nordisk* where the use of a cleavable fusion expression system had not previously been used to express human protein, in this instance, the transformation of plant protoplasts and cells, including monocots, using the methods as described in detail in the application were well known to those of skill in the art (see exemplary references cited above that evidence this), and is described in the application. Further, the Examiner is reminded that the application need not set forth all information necessary to practice the claimed method. MPEP §2164.05(a). Indeed, information that was well known to persons of skill in the art need not be included, and is preferably omitted. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991). In this instance, as discussed in detail above, the specification contains several pages of disclosure regarding transformation of cells, which is inclusive all plant species, including monocots. The Applicant need not detail every step of every method of transforming a cell. Transformation techniques of plant cells was routine at the time of filing this application.

Accordingly, Applicant respectfully submits that it would not require undue experimentation to introduce SATACs into plants cells, including monocots and dicots, to practice the subject matter as claimed to generate a transgenic plant. Methods of plant transformation without undue experimentation are supported by the teachings of the specification, along with the fact that the level of skill and knowledge of those of skill in the art is high, and the prior art describes all methods needed to practice the methods of plant transformation. Further, Applicant's own declarations of record evidence the application of several plant transformation techniques to introduce SATACs into plant cells, including monocots and dicots, based on the teachings of the specification.

2) The specification, in view of other factors, teaches methods of obtaining SATACs from any plant or animal species to sufficiently enable claims to a broad genus

The Examiner appears to urge that Applicant's previous arguments that the disclosure of methods of obtaining SATACs, as set forth in the specification, is not sufficient for the enablement of claims broadly drawn to any SATAC from any animal or plant species. The Examiner cites Bayer v. Housey.

Applicant respectfully submits that the instant application provides sufficient description of SATACs for one of skill in the art to produce a SATAC from any cell type or species of cell, and to identify a SATAC. As discussed in detail above with regard to written description and enablement, SATACs and elements of SATACs are described in detail and exemplified throughout the specification and working examples. SATACs are described by structural features such as centromeres, telomeres, and heterochromatin. The instant application provides description and drawings exemplifying these structures (see, for example, Figures 2 and 3). Thus, one of skill in the art using such teachings could recognize a SATAC. Further, the instant application describes that the generation of SATACs is universal and provides in great detail each of the steps involved in generating a satellite artificial chromosomes, including detailed characterization of the intermediates involved, so that one of skill in the are could produce and identify a SATAC. As discussed in detail with regards to the factors enumerated in In re Wands, the methods taught in the application are applicable to SATACs regardless of species.

Furthermore, the Declarations of record and Declaration 5 and Declaration 6 provided herewith unequivocally demonstrate the universality of the methods. The Declarations demonstrate that by following the teachings of the specification, the methods operate as claimed for the generation of plant SATACs and human SATACs. The Declarations show that the instant application teaches method that are universally applicable to the generation of SATACs in eukaryotes, including the generation of plant SATACs.

Further, Applicant's reliance on Bayer v. Housey (68 USPQ2d 1001, 1008-1009 (Fed. Cir. 2003) is misplaced. The decision in Bayer v. Housey is directed to importation of a product under 35 U.S.C. § 271(g). In this instance, Housey, the assignee of U.S. method patents directed to a process for identifying whether a particular agent can activate or inhibit a target protein, alleged that Bayer infringed the patents by importing into United States a drug derived from the patented process. The court held that infringement is limited to

physical goods manufactured by a patented process, and does not extend to information derived by such a patented process. In addition, Housey alleged that Bayer infringed by selling in the United States a drug containing an activator or inhibitor of a protein identified by Housey's patented method. As to this second theory, the court stated that "the process must be used directly in the manufacture of the product, and not merely as a predicate process to identify the product to be manufactured." Thus, Bayer v. Housey does not address enablement, and is inapt to any facts at issue in the instant prosecution.

3) The Declarations of record and provided herewith evidence the generation of SATACs, including plant SATACs; the introduction of SATACs into plant cells; and the generation of transgenic plants based on the teachings of the specification as of its earliest filing date

The Examiner urges that for reasons of record that Declaration 3 is unpersuasive, Declaration 4 also is unpersuasive. Specifically, the Examiner states that the method of set forth in Declaration 3 and Declaration 4 of Fabijanski are not supported by the specification as of its date of filing because Fabijanski employs a DNA sequence not available until 27 September 1996 at the earliest.

As described above with respect to the Written Description rejection, the earliest filed application filed April 10, 1996 teaches that the generation of a SATAC by introducing a piece of heterologous DNA into a cell, whether by non-targeted or targeted integration, growing the cell, and looking for cells that contain satellite artificial chromosomes. Use of a selectable marker on the piece of heterologous DNA renders it easier to find the cells in which the events that lead to the generation of satellite artificial chromosomes occur. Such cells can them be selected, and a satellite artificial chromosome isolated therefrom. Also, as described in the earliest filed application, the heterologous DNA can be introduced with a sequence that targets it to the pericentric region of the chromosome, which increases the frequency of the recombination and the amplification event, although this is not required.

It is respectfully submitted that each of the Declarations of record evidence the generation of a SATAC based on the above description as taught in the specification. For example, each of Declarations 3 and 4 (and Declaration 2, which is simply a less detailed analysis of the study set forth in Declaration 4), teach the introduction of heterologous DNA into cells, which is increased in the presence of a targeting sequence (the rDNA, not the 1996 APS sequence). In the case of each of Declarations 2, 3 and 4 the heterologous DNA includes a selectable marker and the targeting sequence is an Arabidopsis sequence with homology to the pericentric DNA sequences of the plant chromosome. The application

describes in detail that the frequency of integration of heterologous DNA can be increased by targeting the pericentric heterochromatin by including DNA, such as rDNA, to direct integration to these regions (see page 29, lines 12-21). Also at the time of filing, it was known that rDNA was part of the pericentric heterochromatin in plants. For example, as detailed in Declaration 5 provided herewith, the coding region of the 26S rDNA is highly conserved among species and it encodes a structural RNA molecule highly conserved among eukaryotic organisms. Also, there are multiple sources of rDNA coding sequences available, dating back as early as 1985 (see *e.g.*, Takaiwa *et al.* (1985) Gene, 37:255-9.) Thus, the use of a pericentric rDNA sequence in connection with generation of a plant SATAC is consistent with the teachings of the instant application. Although Declaration 4 also describes other components that are introduced (i.e. the 334 bp tobacco pericentric sequence), these are not required. The 334 bp tobacco sequence is an amplification promoting sequence. It is not part of the method, but is heterologous DNA, how introduction is confirmed. For example, as detailed extensively in the response filed November 09, 2005, the results in Declaration 4 of Fabijanski confirmed that the 334 bp sequence was not required.

Notwithstanding this, the Declaration 5 of Fabijanski provided herewith shows generation of plant satellite artificial chromosomes using DNA fragments that do not include he 334 bp tobacco sequence. Declaration 5 shows that plant SATACs from two diverse plant species were generated by introducing heterologous DNA containing a selectable marker and the 1.7 kb Arabidopsis rDNA described above. The 334 bp tobacco sequence was not included. Thus, the fact that this sequence **may** have been deposited in 1996 after the earliest filing date is irrelevant.

It is respectfully submitted that the Declarations of record describe the generation of SATACs, including plant SATACs, based on the teachings of the specification. Nevertheless, Declaration 4 and Declaration 5 show that plant SATACs can be generated without the 334 bp tobacco sequence, based on the teachings of the specification simply by introducing heterologous DNA. In the studies in the declarations, Dr. Fabijanski chose to include a targeting sequence, and selected a 1.7 kb Arabadopsis sequence known before the earliest priority date of the instant claims. Further, Applicant is not required to teach that which is well known. At the time of filing the application, it was well known that plants, like other eukaryotes, have pericentric heterochromatin, and exemplary rDNA sequences were known. The fact that other pericentric sequences, such as rDNA sequences, are later discovered does not negate the teachings of the specification, and does not preclude one of

skill in the art from using such sequences. Hence, Applicant requests that the Examiner consider all Declarations of record because they do evidence the full scope of the claimed subject matter based on the teachings of the specification.

V. Rejection of Claims 50, 51, 52, 73, 80, 88-92, 94-96, 98-100, 107, 114, 117-118 and 120-122 Under U.S.C. §102(b)

Claims 50, 51, 52, 73, 80, 88-92, 94-96, 98-100, 107, 114, 117-118 and 120-122 are rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Richards *et al.* (U.S. Patent No. 5,270,201, issued 14 December 1993) because Richards *et al.* allegedly discloses a method of making an artificial chromosome, using it to transform plant cells (wherein the plant cell is a protoplast), wherein the artificial chromosome encodes a gene product, wherein the artificial chromosome is introduced by direct DNA transfer, and wherein the plant cell is from a monocot, a dicot or an algae. Therefore, it is concluded in the Office Action that Richards *et al.* anticipates the rejected claims because the rejected claims are alleged not to be drawn to a method of making an artificial chromosome through amplification, nor are these claims drawn to an artificial chromatin which is predominantly heterochromatic. This rejection respectfully is traversed.

Relevant law

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. In re Spada, 15 USPQ2d 1655 (Fed. Cir, 1990), In re Bond, 15 USPQ 1566 (Fed. Cir. 1990), Soundscriber Corp. v. U.S., 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, Richardson v. Suzuki Motor Co., 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." In re Lang, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). It is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co., 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). A reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference In re Oelrich, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

The rejected claims

Independent claim 92 is directed to a method of producing a transgenic plant by a method including steps of:

introducing a **satellite artificial chromosome (SATAC)** into a plant cell; and

growing the plant cell under conditions to produce a transgenic plant.

All other rejected claims are dependent on claim 92.

Hence, the claims specify that a transgenic plant is generated by **introducing a SATAC** into a plant cells. As described in detail in this and all previous responses (incorporated by reference herein), a SATAC is a chromosomal structure first identified and characterized by Applicants. The specification defines a SATAC as:

> As used herein, a SATAC refers to a chromosome that is substantially all heterochromatin, except for portions of heterologous DNA. Typically, SATACs are satellite DNA based artificial chromosomes, but the term encompasses any chromosome made by the methods herein that contains *more heterochromatin than euchromatin.*

. As described in detail in the application, a SATAC is produced by an amplification event upon introduction of heterologous DNA into cells, growing the cell and then selecting those cells that have a SATAC based on its identifying characteristics, such as being predominantly heterochromatic. Accordingly, contrary to the Examiner's assertions, the claims are drawn to a chromosome that is produced by an amplification event, and that is predominantly heterochromatic. This is a SATAC.

Differences between the disclosure of Richards *et al.* and the rejected claim

Richards *et al.* describes the isolation of a telomeric clone from A. thaliana and methods that for obtaining ARS and centromeric sequences from A. thaliana. Example 19, which is prophetic, purports to provide a method for assembling the telomeres, ARS and centromere into an artificial chromosome.

Richards *et al.* does not disclose an artificial chromosome that is a satellite artificial chromosome (SATAC), nor is any method described in which heterochromatin would be amplified, nor does Richards *et al.* disclose selecting cells that contain a SATAC. Richards *et al.* does not have a hint or whiff of disclosure of a SATAC, and, thus, could never disclose a method that includes a step of "introducing a satellite artificial chromosome (SATAC) into a plant cell" to generate a transgenic plant, since Richards *et al.* does not disclose (teach or suggest or hint at) the existence of a SATAC.

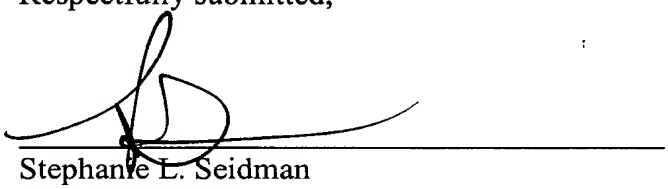
As described in the specification, a SATAC is an artificial chromosome that contains more heterochromatin than euchromatin. Claims are to be read in light of the specification. Richards *et al.* does not disclose an artificial chromosome that contains more heterochromatin

than euchromatin. Therefore, Richards *et al.* fails to disclose all elements as claimed Thus, Richards *et al.* does not anticipate claim 92, nor any claims dependent thereon.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,



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